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LIPID COMPOSITION OF PHOTORECEPTOR MEMBRANES FROM GOLDFISH RETINAS

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Isolation and biochemical characterization of goldfish retinal photoreceptor outer segment membranes are described. The lipid fraction is composed primarily of phospholipids (68 mol%) with substantial amounts of neutral lipids (32 mol%). Sterols account for only about 2 wt% of the membranes (about 9 mol% of the total lipids). The phospholipid class composition and fatty acid composition are similar to those of other vertebrate photoreceptor membranes. Two novel findings were the high levels of free fatty acids (21 mol% of the total lipids, primarily palmitic and docosahexaenoic acids) and the presence of relatively significant amounts of a C-32 diacylglycerol molecular species.

Introduction

The biochemical composition of retinal rod outer segment membranes from a variety of vertebrate retinas has been examined in great detail [1–3]. In general, lipids and proteins each represent approx. 50% of the membrane, by weight. Phospholipids are the major lipid constituents, while the visual pigment rhodopsin accounts for most of the membrane protein. Vertebrate rod outer segment membranes contain very small amounts of sterols (presumably cholesterol) and unusually large amounts of long-chain polyunsaturated fatty acids, especially docosahexaenoic acid (22 : 6 − 3). Free fatty acids, 1,2-diacylglycerols, and lysophospholipids are generally present only as minor membrane components, and triacylglycerols and steryl esters are negligible. Some exceptions to these generalizations have been noted. For example, relatively substantial amounts of sterol (presumably cholesterol) have been detected in the rhabdomeric photoreceptor membranes of cephalopod [4–6] and *Limulus* [7] retinas. Also, it has been reported that lysophospholipids and free fatty acids are substantial components of the rod outer segment lipids of various marine teleost fish retinas [8].

We have been studying the biosynthesis and utilization of photoreceptor membrane phospholipids in rod photoreceptors isolated from goldfish retinas [9]. It was of interest, therefore, to determine the lipid composition of the goldfish photoreceptor membranes. During the course of our studies, we found considerable difficulty in attaining high yields of outer segment membranes free from attached inner segments using conventional methodology. We report here the results of our investigations concerning the isolation and composition of rod outer segment membranes from the retina of the freshwater teleost, *Carassius auratus*.

Methods

Preparation of rod outer segment membranes

Goldfish (*Carassius auratus*), 15–20 cm in length, were obtained from Ozark Fisheries (St. Louis, MO). The fish were maintained in cyclic lighting (12 h dark/12 h light) at 20 ± 1°C (water temperature) for at least 2 weeks prior to use.

Dark-adapted fish were killed within 2 h after the time of normal light onset. All dissections and preparative procedures were carried out at 0–4°C under dim red light. Retinas free of pigment epithelium (eight per group) were homogenized in 5 ml buffer (10 mM Tris-acetate (pH 7.4)/2 mM $MgCl_2$/0.15 mM $CaCl_2$/0.10 mM Na_2EDTA) with a loose ground glass-on-glass tissue grinder (ten passes by hand). Homogenates were transferred to 17-ml polypropylene centrifuge tubes, diluted with buffer to approx. 16 ml, and then centrifuged for 20 min at $27000 \times g$. The supernatants were discarded, and the pellets were resuspended in 4 ml buffer containing 42% (w/w) sucrose. The resuspended material was transferred to 17-ml capacity cellulose nitrate centrifuge tubes and further homogenized with a motor-driven Teflon pestle (15 passes at 1000 rpm). The samples were then sequentially overlayered with 4 ml each of buffered 1.151, 1.133 and 1.113 g/cm^3 sucrose and centrifuged for 90 min at $100000 \times g$ in a swinging bucket rotor. Material which banded at the 1.113/1.133 g/cm^3 density interface was harvested and a portion was processed for electron microscopy (see below). The majority of the membrane material was washed twice with 16-ml portions of buffer (20 min at $45000 \times g$) and once with 16 ml distilled water. The washed membranes were then resuspended in 4.0 ml of distilled water and immediately processed (see below).

Assessment of membrane purity

Unwashed membranes from the 1.113/1.133 g/cm^3 interface were fixed with 2% glutaraldehyde-2% formaldehyde in 0.1 M potassium phosphate buffer (pH 7.4) and post-fixed in 1% OsO_4. After dehydration through a graded ethanol-water series, the membrane material was embedded in Epon. Ultra-thin sections were obtained with a Sorvall MT-2B ultramicrotome and examined with a Siemens IA electron microscope.

A portion of the washed, resuspended membranes was diluted 10-fold with 2% octyl-β-D-glucopyranoside (octyl glucoside; Calbiochem-Behring, La Jolla, CA) in 67 mM phosphate buffer (pH 7.1), and the absorption spectrum was scanned from 700 nm to 250 nm with a Cary 219 spectrophotometer. After bleaching (10 min, ambient room light), the sample was rescanned. The spectral ratio $A_{280}/\Delta A_{522}$ (the absorbance at 280 nm divided by the difference in absorbance at 522 nm before and after bleaching) was determined without correction for light scattering.

Another portion of the membrane suspension (containing 20–60 μg of protein) was analyzed by polyacrylamide disc gel electrophoresis in the presence of sodium dodecyl sulfate [10]. Plots of log M_r of standard proteins versus their mobility relative to a tracking dye were used to determine the apparent molecular weights of photoreceptor membrane proteins.

Compositional analyses

Total protein was determined by the method of Lowry et al. [11], using bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as a standard. Membranes were solubilized with 1 M NaOH (16 h at 65°C) prior to protein determination.

Membrane suspensions were extracted twice with 5 vol. chloroform/methanol (2 : 1, v/v) and partitioned by centrifugation. The chloroform phases were combined, and solvent was removed at room temperature under a stream of nitrogen. Residues were immediately redissolved in 1.0 ml of chloroform and aliquots were removed for phosphorus analysis [12]. Neutral lipids were separated from phospholipids and fractionated by one-dimensional thin-layer chromatography on glass plates coated with Silica-Gel G using a solvent system of hexane/diethyl ether/glacial acetic acid (70 : 30 : 1, v/v). Authentic standards of cholesterol, diolein, oleic acid, triolein, methyl oleate, and cholesteryl oleate (Sigma) were chromatographed in an adjacent lane. Phospholipids were analyzed by two-dimensional thin-layer chromatography as previously described [13]. The 1,2-diacylglycerols were isolated, acetylated and analyzed as described in detail by Wiegand and Anderson [14,15]. Methyl esters of free fatty acids, phospholipids and total lipids were prepared with BF_3/MeOH and quantitatively analyzed as previously described [4,14,15].

Results

Purity of goldfish photoreceptor membrane preparations

Electron microscopy of material from the

1.113/1.133 g/cm^3 sucrose density interface (Fig. 1) showed the presence of fragmented photoreceptor outer segments (stacks of membrane lamellae) as well as numerous single-walled vesicles (presumably free discs). Also present were extended linear aggregates of vesicles apparently attached to a central, electron-dense, ribbon-like structure (see open arrows, Fig. 1). These structures are similar in appearance to the 'vesiculated membrane structures' observed by Saari and Bunt [16] in preparations of rabbit, bovine, monkey and goldfish photoreceptor membranes. The cell type from which

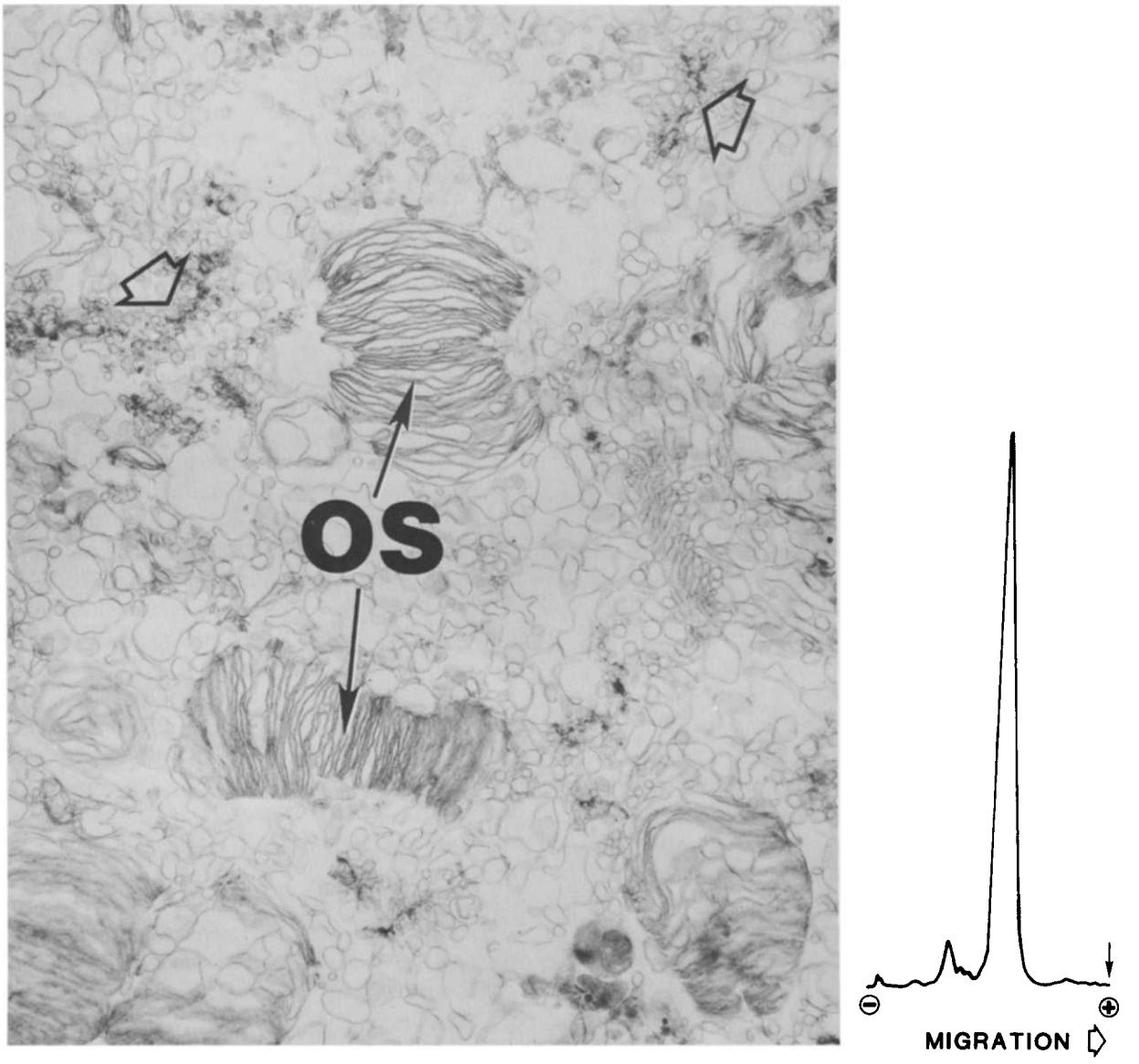

Fig. 1. Electron micrograph of photoreceptor membranes prepared from goldfish retinas. OS, partially intact outer segments. Note the presence of numerous single-walled vesicles, and vesicle aggregates (open arrows). ×23000.

Fig. 2. Densitometer tracing of a polyacrylamide gel (Coomassie blue stained) of solubilized goldfish photoreceptor membranes. The major peak corresponds to a component having an apparent M_r of 39200. The arrow corresponds to the position of the tracking dye. Gel length, 9.1 cm. Total applied protein, 35 μg.

these vesiculated structures originate is not known with certainty, although they are thought to be derived from cone outer segments [16]. Other identifiable cellular organelles were not observed in our membrane preparations.

Photoreceptor membrane preparations solubilized in buffered octyl glucoside exhibited the characteristic spectroscopic properties of goldfish porphyropsin [17,18], with λ_{max} at 280 nm and 522 nm. The values for the spectral ratio $A_{280}/\Delta A_{522}$ ranged from 3.5 to 3.8. The observed values are probably higher than the true values, since goldfish porphyropsin is relatively thermally unstable in octyl glucoside solutions [19] (resulting in a loss of absorbance at 522 nm), and the spectra were not corrected for light scattering (resulting in an elevated absorbance at 280 nm). Also, these values are inherently higher than the spectral ratios obtained for photoreceptor membranes containing rhodopsin (average $A_{280}/\Delta A_{500}$ values approx. 2.0–2.5) due to the fact that the maximum photosensitivity of porphyropsin is only about 75% that of rhodopsin [20].

Polyacrylamide gel electrophoresis of detergent-solubilized photoreceptor membrane preparations indicated the presence of only one major protein component, having an apparent M_r of 39200 ± 1900 ($n = 6$). A similar value for the apparent molecular weight of goldfish opsin (38000) has been reported by Bunt and Saari [24]. In the densitometer scan shown in Fig. 2, this component represents at least 85% of the total Coomassie-blue-staining material on the gel. Proteins of lower molecular weight were negligible. The absolute number and relative amount of other stainable components on the gels varied from one preparation to the next, and most of these appeared to be aggregates of the 39.2 kDa component. These observations are consistent with the known behavior of vertebrate opsins on polyacrylamide gel systems of the type employed here [1,21,22].

Taken together, these data indicate that the photoreceptor membrane preparations used throughout this study were of relatively high purity and homogeneity, based on the criteria commonly accepted for other vertebrate photoreceptor membranes [1–3,21,22].

Composition of goldfish photoreceptor membranes. Goldfish photoreceptor membranes contain 64.7 ± 5.8 wt% protein and, by difference, 35.3 ± 3.2 wt% lipid. Peters et al. [23] have shown that quantitation of opsin by the method of Lowry et al. [11] using bovine serum albumin as a standard yields a value which is 1.29-times greater than that obtained by quantitative amino acid analysis. Adjusting our data accordingly gives a protein/lipid weight ratio of approx. 1 : 1. On a weight basis, about 83% of the lipid is phospholipid and 17% is neutral lipid. Sterols account for only 2.3 ± 0.6 wt% of the membranes, in general agreement with the results obtained for the rod outer segment membranes of other vertebrates [1–3,22]. Although the sterol component of the lipid extracts comigrated with cholesterol on gas-liquid chromatography, we cannot definitively identify this component as cholesterol without further, more rigorous chemical and chromatographic analyses (see Ref. 22).

The lipid composition of goldfish photoreceptor membranes is given in Table I. On a molar basis, phospholipids account for about 65% and neutral lipids about 32% of the total membrane lipids. The major phospholipid classes are phosphatidylcholine (34 mol%), phosphatidylethanolamine (21 mol%), and phosphatidylserine (6 mol%). Small amounts of phosphatidylinositol, sphingomyelin and 1,2-diacylglycerols were also detected. We did not detect appreciable amounts of phosphatidic acid, lysophospholipids, triacylglycerols or steryl esters in these membrane preparations. Sterols (9 mol% of total lipid) account for about 30% of the neutral lipids. A striking feature of the lipid composition data is the unusually high level of free fatty acids, which account for 21 mol% of the total lipids, or about two-thirds of the neutral lipids.

A comparison of the phospholipid class composition of goldfish retina lipids and photoreceptor membrane lipids is given in Table II. The phospholipid class compositions are very similar, with retina containing somewhat higher levels of phosphatidylcholine and lower levels of sphingomyelin and phosphatidylserine. Only small amounts of phosphatidylinositol were detected in both whole retina and photoreceptor membrane lipids, and lysophosphatides were notably absent (see Discussion).

TABLE I

LIPID COMPOSITION OF GOLDFISH PHOTORECEPTOR MEMBRANES

Values expressed as the means ± S.D. of single determinations from seven independent preparations. n.d., not detected.

Lipid class	Relative mol%
Glycerolipids	
Glycerophospholipids (GPL)	
Phosphatidylcholine	34.0 ± 3.4
Phosphatidylethanolamine	20.6 ± 0.7
Phosphatidylserine	6.1 ± 0.5
Phosphatidylinositol	1.1 ± 0.8
Lysophosphatidylcholine	n.d.
Lysophosphatidylethanolamine	n.d.
Unknown	3.3 ± 1.3
Neutral glycerolipids (NGL)	
1,2-Diacylglycerols	1.3 ± 1.0
Triacylglycerols	n.d.
Sphingolipids (SL)	
Sphingomyelin	3.1 ± 0.5
Free fatty acids (FFA)	21.0 ± 3.2
Sterol (S)	9.4 ± 1.3
Steryl esters	n.d.
Total phospholipids (GPL + SL)	68.2
Total Neutral Lipids (NGL + FFA + S)	31.7

The fatty acid composition of the total lipids from goldfish retina and photoreceptor membranes is presented in Table III. On a relative mol% basis, the retina lipids have higher levels of saturated and monounsaturated fatty acids (especially 16 : 0, 18 : 0, and 18 : 1 species) and substantially lower levels of polyunsaturated fatty acids (primarily 22 : 6 − 3) than photoreceptor membrane lipids. More than two-thirds of the photoreceptor membrane fatty acids are polyunsaturated, primarily due to the high levels of 22 : 6 − 3 (69 mol%). A distinct lack of appreciable amounts of dimethylacetals was noted, indicating that plasmalogens are not present in significant amounts in either goldfish retina or photoreceptor membranes.

The fatty acid composition of the major phospholipid classes and free fatty acids of goldfish photoreceptor membranes is presented in Table IV. Of the three major phospholipid classes, phosphatidylethanolamine contains the highest levels

TABLE II

PHOSPHOLIPID CLASS COMPOSITION OF GOLDFISH PHOTORECEPTOR MEMBRANES AND WHOLE RETINAS

Values are means ± S.D. of duplicate determinations from three (membranes) or four (retinas) independent preparations. Recovery of applied lipid phosphorus, 98%. Abbreviations: Sph, sphingomyelin; PI, phosphatidylinositol; PS, phosphatidylserine, PC, phosphatidylcholine; PE, phosphatidylethanolamine.

Class	Relative mol%	
	Membranes	Retina
Sph	4.5 ± 0.7	1.5 ± 0.6
PI	1.6 ± 1.2	1.0 ± 0.6
PS	8.9 ± 0.8	5.9 ± 1.5
PC	49.9 ± 5.0	57.7 ± 2.3
PE	30.2 ± 1.0	32.7 ± 1.7
Unknown	4.8 ± 1.9	1.3 ± 0.4

TABLE III

FATTY ACID COMPOSITION OF THE TOTAL LIPIDS OF GOLDFISH PHOTORECEPTOR MEMBRANES AND WHOLE RETINAS

Values are means ± S.D. of single determinations from four (membranes) or five (retinas) independent preparations. DMA, dimethyl acetals formed by acid methanolysis of plasmalogens.

Fatty acid	Relative mol%	
	Membranes	Retina
14 : 0	0.2 ± 0.2	0.7 ± 0.3
15 : 0	0.1 ± 0.1	0.1 ± 0.1
16 : 0 DMA	0.3 ± 0.1	0.3 ± 0.3
16 : 0	9.1 ± 2.1	16.7 ± 2.0
16 : 1	1.8 ± 0.6	2.0 ± 0.6
17 : 0	0.3 ± 0.1	0.4 ± 0.2
18 : 0 DMA	0.3 ± 0.1	0.4 ± 0.3
18 : 0	10.5 ± 0.3	18.7 ± 4.4
18 : 1	4.8 ± 1.9	8.5 ± 1.5
18 : 2 − 6	0.3 ± 0.1	0.6 ± 0.4
20 : 0	0.1 ± 0.0	0.1 ± 0.0
20 : 1 − 9	0.4 ± 0.1	0.8 ± 0.2
20 : 2 − 6	0.3 ± 0.1	0.8 ± 0.7
20 : 3 − 9	0.1 ± 0.0	0.1 ± 0.0
20 : 4 − 6	0.6 ± 0.1	1.2 ± 0.2
20 : 5 − 3	0.1 ± 0.0	0.5 ± 0.1
22 : 4 − 6	0.1 ± 0.1	0.1 ± 0.1
22 : 5 − 6	0.5 ± 0.1	0.6 ± 0.1
22 : 5 − 3	0.5 ± 0.1	0.8 ± 0.1
22 : 6 − 3	69.4 ± 4.9	45.5 ± 4.8
% Polyunsaturates	71.3	48.9
% Saturates	20.2	36.6

TABLE IV

FATTY ACID COMPOSITION OF THE MAJOR PHOSPHOLIPID CLASSES AND FREE FATTY ACIDS OF GOLDFISH PHOTORECEPTOR MEMBRANES

Values expressed as the mean ± S.D. of single determinations from six independent preparations. PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; FFA, free fatty acids. DMA, dimethyl acetals formed by acid methanolysis of plasmalogens.

Fatty Acid	Relative mol%			
	PS	PE	PC	FFA
14:0	0.9±0.3	0.3±0.1	1.1±0.2	3.1±0.6
15:0	0.2±0.1	0.2±0.2	0.3±0.1	1.0±0.2
16:0 DMA	0.2±0.1	1.1±0.9	0.2±0.1	–
16:0	9.6±1.9	6.4±1.3	29.7±1.7	21.1±3.2
16:1	2.2±0.6	2.5±1.1	5.0±0.3	3.9±0.9
17:0	1.0±0.2	0.4±0.2	0.5±0.1	4.5±0.7
18:0 DMA	0.2±0.1	0.7±0.5	0.3±0.1	0.5±0.2
18:0	27.5±3.7	13.4±1.0	14.6±1.8	14.6±1.3
18:1	5.1±0.6	8.1±1.6	17.7±0.8	9.3±2.9
18:2	1.3±0.4	0.9±0.21	1.2±0.3	2.3±0.1
20:0	0.4±0.1	0.2±0.2	0.1±0.1	3.9±2.0
20:1	1.0±0.3	1.1±0.2	1.1±0.1	1.4±0.4
20:3	0.4±0.1	0.3±0.1	0.3±0.1	–
20:4	1.3±0.4	2.4±0.6	1.2±0.3	2.0±0.3
20:5	0.2±0.1	0.5±0.1	0.4±0.2	0.8±0.2
22:4−6	2.6±1.7	0.4±0.4	0.5±0.3	9.3±1.8
22:5−6	1.0±0.4	0.7±0.3	0.7±0.2	0.7±0.1
22:5−3	1.7±0.3	1.2±0.2	0.4±0.1	0.8±0.3
22:6−3	42.3±5.9	58.0±4.9	24.4±2.4	20.6±3.3
% Polyunsat.	49.5	63.5	27.9	38.1
% Saturates	39.2	20.7	45.7	44.3

of polyunsaturated fatty acids (64 mol%) and relatively low levels of saturated fatty acids (21 mol%). The reverse is true for phosphatidylcholine, where 28 mol% of the acyl chains are polyunsaturated and 46 mol% are saturated. The acyl chain composition of phosphatidylserine is intermediate between these two extremes (50 mol% polyunsaturated, 39 mol% saturated). Each phospholipid class has certain distinctive features of composition. Phosphatidylethanolamine has relatively high levels of 22:6 − 3 (58 mol%) and moderate levels of 18:0 (13 mol%). Phosphatidylcholine contains relatively high levels of 16:0 (30 mol%) and 18:1 (18 mol%) and relatively moderate levels of 22:6 − 3 (24 mol%) and 18:0 (15 mol%). Phosphatidylserine has relatively high levels of 18:0 (28 mol%) and 22:6 − 3 (42 mol%). The finding that the levels of 22:6 − 3 are higher in the total lipids (cf. Table III) than in any phospholipid class of the photoreceptor membrane can be accounted for by the extreme lability of the polyunsaturates (especially 22:6 − 3) to oxidative degradation during the procedures used to isolate each phospholipid class for analysis. Hence, the values given for polyunsaturated fatty acids from a given phospholipid class represent minimum values, whereas those given saturated, monounsaturated and diunsaturated fatty acids represent maximum values. Although the composition of the free fatty acids most closely resembles the acyl chain composition of phosphatidylcholine, the free fatty acids contain significantly lower levels of 18:1 and higher levels of 17:0, 18:3 and 22:4 − 6. The free fatty acids are predominantly saturated (44 mol%), owing largely to the relatively substantial amounts of 16:0 and 18:0, and the relatively moderate amounts of 22:6 − 3. In comparison with the total lipids of whole retina and photoreceptor membranes (see Table III), the free fatty acids have higher levels of 18:2, 18:3 − 3, 20:4 − 6 and 22:4 − 6, but considerably lower levels of 22:6 − 3. These results are generally consistent with the data obtained for the free fatty acid composition of frog [15], rat [15] and bovine [30] photoreceptor membranes.

Diacylglycerols account for only about 1 mol% of the total lipid (or about 4% of the neutral lipids) of goldfish photoreceptor membranes. The composition of photoreceptor 1,2-diacylglycerols, expressed as the percentage distribution according to

TABLE V

MOLECULAR SPECIES DISTRIBUTION OF DIACYLGLYCEROLS IN PHOTORECEPTOR MEMBRANES

For goldfish, values are means ± S.D. of single determinations from eight independent preparations. For frog and rat, data are of Wiegand and Anderson [15].

Carbon number	Relative mol%		
	Goldfish	Frog	Rat
32	13.3±3.9	–	–
34	13.2±2.3	3	8
36	7.1±2.4	3	28
38	22.9±3.1	52	60
40	43.5±3.8	42	3

carbon number, is given in Table V. For goldfish, the major molecular species are C-40 (44%) and C-38 (23%), with lesser amounts of C-32 (13%), C-34 (13%), and C-36 (7%) species. The data of Wiegand and Anderson [15] for the diacylglycerol distribution in frog and rat photoreceptor membranes are included in Table V for comparison. There is considerable phylogenetic variability apparent. The frog membranes contain almost exclusively the C-38 and C-40 molecular species, whereas the rat membranes contain primarily the C-38 and C-36 diacylglycerols. Neither frog nor rat photoreceptor membranes contain detectable amounts of the C-32 diacylglycerol, in contrast to the data obtained for goldfish photoreceptor membranes.

Discussion

Prior to this study, no detailed report of the preparation and composition of goldfish photoreceptor membranes has appeared in the literature. Our approach to the preparation of the photoreceptor membranes was essentially that of Papermaster and Dreyer [21], with some modifications. The association between retina and vitreous in the goldfish eye is quite tenacious; hence, we routinely homogenize dark-adapted goldfish retinas in buffer and sediment the retinal material to remove adhering vitreous prior to fractionation of the retina by discontinuous sucrose density centrifugation. This method also resulted in freeing the photoreceptor outer segments from inner segments. Approx. 60–75% of the bleachable material on the gradients banded at the 1.113/1.133 g/cm^3 sucrose density interface, while the remainder banded at the 1.133/1.15 g/cm^3 interface. Although the data indicate that the material harvested from the 1.113/1.131 g/cm^3 interface is highly enriched in photoreceptor membranes, we cannot ascertain what proportion of the material is derived from rods and what proportion is from cones. Although their methodology for isolating outer segment membranes was not described in detail, Bunt and Saari [24], have noted a similar distribution of goldfish photoreceptor membranes on their sucrose density gradients and have obtained some evidence which suggests that, although rod outer segment membranes are enriched in the 1.11/1.13 g/cm^3 interfacial material and cone outer segment membranes are enriched in the 1.13/1.15 g/cm^3 interfacial material, both interfacial layers contain material derived from both rods and cones. Considering the details of our preparative procedures and our results (electron micrographs, absorption spectra, and polyacrylamide gels), the results of Bunt and Saari [24], and the fact that at least 85% of goldfish retinal photoreceptors are rods [25,26], we conclude that the material referred to throughout this study as 'photoreceptor membranes' is highly enriched in rod outer segment membranes, but probably contains some cone outer segment membranes as well.

The lipid class composition of goldfish photoreceptor membranes is similar in many ways to that of other vertebrate photoreceptor membranes (reviewed in Ref. 1–3). However, the phospholipid/neutral lipid ratio of goldfish photoreceptor membranes is more like that of the rhabdomeric photoreceptor membranes of invertebrates, such as cephalopods [4–6], than of vertebrate photoreceptor membranes. In the invertebrate systems so far studied [2,4–7], the high neutral lipid content of the photoreceptor membranes has been attributed to their relatively high levels of sterol (about 17 mol% of the total lipids of octopus [4], squid [5], and *Limulus* [7] photoreceptor membranes, and almost 30 mol% of the total lipids of *Eledone* photoreceptor membranes [6]). The mole ratio of sterol/free fatty acids in the rhabdomeric photoreceptor membrane is usually about 2 : 1 [2,4,5,7], but can be nearly 4 : 1 [6]. In goldfish photoreceptor membranes, however, this ratio is reversed, and free fatty acids account for at least 20 mol% of the total lipid. This level of free fatty acids is the highest found so far for photoreceptor membranes. Although high levels of free fatty acids have been reported for photoreceptor membranes of other teleost fish [8], the concomitantly high levels of lysophospholipids in those preparations suggest to us that a significant portion of the free fatty acids may have been generated by phospholipase A_2 activity during the preparation of those membranes. Our extensive lipid analyses failed to detect the presence of appreciable amounts of lysophospholipids in either goldfish photoreceptor membranes or whole retinas. Furthermore, detailed fatty acid analyses indicated that the free fatty acid composition did not correspond to that

of the total lipids of either whole retinas or photoreceptor membranes, nor did the composition closely resemble that of the major phospholipid classes of the photoreceptor membranes. These data suggest that free fatty acids are endogenous constitutents of goldfish photoreceptor membranes and are not degradation products derived from photoreceptor or whole retina phospholipids.

Potentially, the high level of free fatty acids and absence of lysophospholipids could be explained by a sequential degradation of phospholipids by phospholipase A_2 and lysophospholipase. Although enzymatic activities ascribed to phospholipases A_1 and A_2 have been found in bovine retina [27], the conditions of our preparative procedures (i.e., 0–4°C, low levels of Ca^{2+}, presence of EDTA, and pH 7.4) and the fact that isolated photoreceptor membranes possess little, if any, phospholipase A activity [27] would not favor such an hypothesis.

It is difficult to explain the presence of such large amounts of free fatty acids within the framework of current concepts of membrane structure. However, this finding may explain some observations concerning the relative thermal stabilities of vertebrate visual pigments, Firstly, the thermal stability of goldfish porphyropsin in a variety of detergents is considerably lower than that of visual pigments from several other vertebrate species [19]. Secondly, it has been demonstrated that the addition of certain exogenous fatty acids to photoreceptor membrane suspensions or treatment of the membranes with phospholipase A_2 (thereby generating lysophosphatides and free fatty acids) leads to a decrease in thermal stability of rhodopsin, whereas addition of lysophosphatidylcholine alone has no such effect [28]. Thirdly, it has been suggested that the observed variability in the thermal stability of marine teleost fish rhodopsins may be a consequence of differences in the lipid composition of their photoreceptor membranes, which reflects a biochemical adaptation at the membrane level to environmental temperature [29]. Lower environmental temperatures have been correlated with an increase in the degree of unsaturation of the photoreceptor membrane phospholipids (particularly the levels of 22 : 6 − 3) and a decreased thermal stability of rhodopsin [8,29]. Finally, the phospholipid class composition and degree of unsaturation of goldfish (poikilotherm) photoreceptor membranes are not significantly different from those of homiothermic animals, such as cattle, rats and humans (see Ref. 1–3). Therefore, the relative thermal instability of goldfish porphyropsin may be a consequence of the unusually high levels of free fatty acids in the membranes. Furthermore, there is a novel possibility that the high free fatty acid levels may reflect an environmental adaptation which is an alternative to altering the degree of unsaturation of the membrane phospholipids.

The differences in the diacylglycerol molecular species composition of photoreceptor membranes from goldfish, frog and rat retinas are most striking. Since the fatty acid composition of the goldfish diacylglycerols has not been determined, it is not possible to specify the fatty acid pairs for a given diacylglycerol molecular species. Such assignments have been made, however, for the diacylglycerol molecular species of frog and rat photoreceptor membranes [15]. For frog, the major fatty acid components of the C-38 species are 16 : 0 and 22 : 6 − 3, while for rat, the C-38 species is primarily 18 : 0 and 20 : 4 − 6. The C-40 diacylglycerol of frog photoreceptor membranes is primarily 18 : 0 and 22 : 6 − 3, while the C-36 species of rat photoreceptor membranes is primarily 16 : 0 and 20 : 4 − 6. An interesting difference between the diacylglycerol composition of goldfish photoreceptor membranes and that of frog and rat photoreceptor membranes is the presence of the C-32 molecular species (13 mol%) in the goldfish membranes and its absence in the frog and rat membranes. Since the total lipids and phospholipids of the goldfish photoreceptor membranes contain large amounts of 16 : 0 and very little 14 : 0, 15 : 0, or 16 : 1, it is most likely that the C-32 species contains primarily two molecules of 16 : 0. The presence of such a species (containing two saturated fatty acids esterified in the same glycerolipid) would be relatively novel, but has yet to be confirmed. The compositional differences may reflect phylogenetic differences in retinal lipid metabolism of fish, amphibians and mammals, where the divergence in composition is determined by enzymatic specificities related to the composition of the molecules which serve as the immediate substrates for the formation of the diacylglycerols. Based on compositional and metabolic studies

(discussed in Ref. 15), it has been proposed that the photoreceptor diacylglycerols in frogs and rats arise from directed lipolysis of phosphatidylinositol (i.e., by a phosphatidylinositol-specific phospholipase C), rather than by de novo biosynthesis via phosphatidic acid. In the present study, the C-32 diacylglycerol would most likely be derived from phosphatidylcholine, since this phospholipid class contains the highest levels of 16:0. The molecular species variations in the diacylglycerol populations from one phylogenetic species to another may also reflect the relative specificities of the phospholipases for the fatty acid composition of the substrate pools accessible to the phospholipases. Further experiments will be necessary in order to confirm this implied relationship between molecular composition and metabolism.

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