Molecular species of phosphatidylcholine, -ethanolamine, -serine, and -inositol in microsomal and photoreceptor membranes of bovine retina

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Abstract The molecular species compositions of phosphatidylcholine, -ethanolamine, -serine, and -inositol in microsomes and rod outer segments from bovine retina were studied by means of argentation thin-layer chromatography (TLC) after conversion of the phospholipids to labeled acetyldiacylglycerols. A highly unsaturated group of molecular species, called "supraenes" to indicate the presence of more than six double bonds per molecule, was present in all four glycerophospholipids. Docosahexaenoic acid was the major component of these species, in combination with other unsaturated fatty acids, predominantly polyunsaturates. The presence of species containing docosahexaenoate in both positions of the glycerol backbone was suggested by the high percentage (95%) of these fatty acids in the most polar fraction of phosphatidylcholine. In rod outer segments, supraenes were the major component of phosphatidylserine (51% of the molecular species), followed by phosphatidylcholine (31%), phosphatidylethanolamine (21%), and phosphatidylinositol (9%). Hexaenes, which contained docosahexaenoate and saturated fatty acids, predominated in phosphatidylethanolamine (67%), followed by phosphatidylserine and phosphatidylcholine (about 36%), and phosphatidylinositol (12%). Tetraenes made up half the phosphatidylinositols, but amounted to less than 5% of the other glycerophospholipids. Disaturated and monoenoic species made up 14 and 6%, respectively, of phosphatidylcholine. In microsomes, there was less of the docosahexaenoate-containing species and more of the saturated to tetraenoic species. Monoenes predominated in phosphatidylcholine (35%), tetraenes in phosphatidylinositol (71%), and hexaenes in phosphatidylethanolamine and phosphatidylserine (50 and 42%). Supraenes amounted to less than 15% of each of the four glycerophospholipids in microsomes.—**Aveldaño, M. I., and N. G. Bazán.** Molecular species of phosphatidylcholine, -ethanolamine, -serine, and -inositol in microsomal and photoreceptor membranes of bovine retina. *J. Lipid Res.* 1983. **24:** 620–627.

Supplementary key words argentation TLC • dipolyunsaturated species • hexaenes • docosahexaenoic acid

Retinal membranes, particularly those of visual cells, contain the most highly unsaturated glycerophospholipids found in vertebrate tissues. Docosahexaenoic acid (22:6, n-3) is known to be the major polyenoic fatty acid of retinal lipids. Studies on the distribution of the molecular species of major phospholipids in the whole retina of the toad have demonstrated high proportions of molecular species, called "supraenes," that are more unsaturated than the hexaenes (1). These species contain large amounts of docosahexaenoate and are not found in toad brain glycerophospholipids.

Subsequent work has shown that supraenes are also present in the bovine retina (2). Silicic acid TLC subfractionation of phosphatidylcholine, -ethanolamine, and -serine (PC, PE, and PS) (1) from bovine rod outer segments separates three major subclasses, containing saturated-saturated, saturated-unsaturated, and unsaturated-unsaturated fatty acid combinations (3). The presence of dipolyunsaturated species was inferred from the high proportion of polyenoic fatty acids in the unsaturated-unsaturated fraction.

In this study, we report the molecular species composition of phosphatidylcholine, -ethanolamine, -serine, and -inositol in whole bovine retina, rod outer segments, and microsomes, after conversion of glycerophospholipids to acetyldiglycerides. These nonpolar derivatives allow better resolution into species by argentation TLC than could be attained with intact phospholipids. Highly sensitive quantitation of species (even minor glycerophospholipids such as phosphatidylinositol) is also possible with the use of labeled acetic anhydride in the preparation of acetyldiglycerides (4).

Abbreviations: PC, PE, PS, and PI, phosphatidylcholine, -ethanolamine, -serine, and -inositol, respectively; ROS, rod outer segments; GLC, gas-liquid chromatography; TLC, thin-layer chromatography; fatty acids are abbreviated by convention, number of carbon atoms:number of double bonds.

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MATERIALS AND METHODS

Bovine eyes were obtained from a local abattoir and packed in crushed ice for transport to the laboratory. Dissection of the retinas was performed in dim light.

Rod outer segments were detached as described by Shichi et al. (5), and purified according to the method of Papermaster and Dreyer (6). The microsomal fraction was obtained as described elsewhere (4), except that the homogenization was done in an ice-cooled homogenizer and the mitochondrial fraction was not washed.

Separation of phospholipids

Lipid extracts from rod outer segments, microsomes, and whole retina were prepared and washed according to the method of Folch, Lees, and Sloane Stanley (7). Phospholipids were resolved by preparative two-dimensional TLC (8) with the solvents described by Rouser, Fleischer, and Yamamoto (9). When the resolution of serine and inositol glycerophospholipids was not satisfactory, especially in the ROS extracts where the ratio of PS to PI is about 10:1 (10), the lipids were eluted and rechromatographed (11).

Preparation of acetyldiglycerides

Lipids were eluted (12) and taken to dryness under N_2. Choline glycerophospholipids were hydrolyzed by *C. welchii* phospholipase C (Type I, Sigma Chemical Co., St. Louis, MO), essentially according to the method of Parkes and Thompson (13). Ethanolamine, serine, and inositol glycerophospholipids were hydrolyzed in the presence of sphingomyelin by adding 10–20 μl of *B. cereus* phospholipase C (Type V, in $[NH_4]_2SO_4$ solution (Sigma Chemical Co) to each sample of lipid suspended in phosphate buffer (14). Samples were hydrolyzed overnight in the presence of N_2 with constant agitation. The diglycerides were extracted with ether and separated on thin layers of boric acid-impregnated silica gel G (10% w/w) using hexane–ether–acetic acid 35:65:1.5 (by vol). Acetylation was performed essentially according to the method of Banschbach, Geison, and O'Brien (4). [1-^{14}C]Acetic anhydride (27 μCi/nmol) or [^{3}H]acetic anhydride (50 mCi/nmol) (New England Nuclear, Boston, MA) diluted with unlabeled, twice-distilled acetic anhydride was used. Labeled acetyldiglycerides were purified by TLC on silica gel G with hexane–ether 80:20 (by vol). Retinal phosphatidylcholine was used to prepare unlabeled acetyldiglycerides for use as carriers for small lipid samples.

Argentation TLC

Acetyldiglycerides were spotted under N_2 on $AgNO_3$-impregnated silica gel G plates (20% w/w) previously activated at 110°C for 2–3 hr. Polyenoic molecular species were resolved using chloroform–methanol–water 90:10:1 (by vol). Trienes to saturates, running together near the solvent front, were eluted (12) and subjected to another TLC separation on similarly prepared plates developed in chloroform–methanol 99:1 (by vol).

Liquid scintillation counting

After development, plates were sprayed with 0.05% dichlorofluorescein in methanol–water 1:1 (15), and viewed under ultraviolet light. The bands were scraped into vials and suspended in 1 ml of 2 M NaCl. Ten ml of Aquasol-2 (New England Nuclear) was added, and the solutions were mixed thoroughly. The samples were allowed to stand overnight to settle the AgCl and silica. Radioactivity was measured an LSC-250 Beckman spectrometer. Counting efficiency was determined from quenching curves prepared with [^{3}H]toluene or [^{14}C]toluene standards (New England Nuclear). The standards were added to vials containing 2 M NaCl, Aquasol-2, and various quantities of scrapings from $AgNO_3$-impregnated silica gel plates that had been previously activated, run, and sprayed with dichlorofluorescein.

Gas–liquid chromatography

Unlabeled acetyldiglycerides prepared from rod outer segment PC and PE were resolved with chloroform–methanol–water 85:15:1 (by vol). Trienes to saturates were eluted and resolved with chloroform–methanol 99:1 (by vol). The bands were scraped, eluted, and washed (12). The solvents were evaporated, and the samples were methanolyzed (16). Aliquots from phosphatidylinositol and phosphatidylserine were also treated in a similar manner and analyzed by GLC. A Varian Aerograph 1700 gas chromatograph equipped with a stainless steel column packed with 6% diethylene glycol succinate (Hewlett Packard) was used. Methyl esters were identified by their retention times and by hydrogenation (17).

RESULTS

The molecular species composition of PC, PE, PS, and PI in whole retina, microsomes, and rod outer segments is shown in **Tables 1–4.** The fatty acid composition of major molecular species of rod outer segment PC and PE is presented in **Tables 5 and 6,** respectively.

A common characteristic of retinal glycerophospholipids was the presence of a group of molecular species more unsaturated than the hexaenes. The whole fraction was called "supraenes" because it included com-

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TABLE 1. Molecular species of bovine retina phosphatidylcholine

Species	Whole Retina (3)	Microsomes (6)	ROS (5)
		mol %	
Supraenes			
I[a]	1.5 ± 0.1	1.1 ± 0.2	3.3 ± 0.6
II	2.1 ± 0.1	1.3 ± 0.3	9.0 ± 0.3
III	2.6 ± 0.1	1.6 ± 0.3	12.7 ± 0.7
IV	0.9 ± 0.0	0.7 ± 0.1	2.8 ± 0.2
V	2.5 ± 0.2	1.9 ± 0.6	1.6 ± 0.1
VI	—	—	1.8 ± 0.3[b]
Total	9.5 ± 0.3	6.5 ± 1.0	31.1 ± 1.3
Hexaenes	20.8 ± 0.7	17.6 ± 1.0	35.5 ± 1.1
Pentaenes			
I	3.2 ± 0.2	3.3 ± 0.5	4.5 ± 0.2
II	0.9 ± 0.1	1.5 ± 0.2	0.7 ± 0.1
Tetraenes			
I	0.9 ± 0.1	2.1 ± 0.7	0.8 ± 0.1
II	7.4 ± 0.2	8.4 ± 0.7	4.1 ± 0.5
Trienes	2.4 ± 0.1	1.8 ± 0.1	1.2 ± 0.1
Dienes			
I	4.7 ± 0.2	2.1 ± 0.1	1.1 ± 0.1
II		2.4 ± 0.5	0.4 ± 0.1
Monoenes	28.4 ± 0.4	34.6 ± 2.4	6.7 ± 0.7
Saturates	22.5 ± 0.6	17.1 ± 0.7	13.9 ± 0.7

Choline glycerophospholipids were isolated by TLC, hydrolyzed to diglycerides, and acetylated with [1-^{14}C]acetic anhydride. Acetyldiacylglycerols were isolated by TLC and resolved by argentation TLC. Values are means ± SD from the number of samples given in parentheses.

[a] The division into subspecies is based on the bands seen after argentation TLC.

[b] This species was on the tail of hexaenes.

binations of monoenoic fatty acids with 22:6 (hexaenes) and dipolyunsaturated species (Table 5). Supraenes were highly concentrated in rod outer segments (Tables 1–4) but also made up a significant portion of microsomal phospholipids. The molecular species composition of lipids from whole retina resembled that of microsomes. In contrast, rod outer segment lipids contained smaller percentages of species with up to five double bonds and higher percentages of supra- and hexaenes. Supraenes represented one-third of the PCs, 20% of the PEs, half the PSs, and 9% of the PIs in these membranes. The ratio of supraenes to hexaenes in all four glycerophospholipids was higher in rod outer segments than it was in microsomes or in whole retina.

Up to six molecular species were separated from the supraenoic fraction of rod outer segment PC by TLC (Table 1). In terms of the fatty acid composition of these species (Table 5), the most polar fraction, which remains at the origin of the plates, was composed entirely of hexaenoic fatty acids. Thus, about 10% of the supraenoic PCs was made up of dodecaenoic molecular species, predominantly didocosahexaenoyl-PC. The presence of molecular species containing eleven, ten, etc., double bonds in the other fractions could be inferred from the possible combinations of 22:6 with other unsaturated fatty acids. However, the amount of 22:6 in these fractions exceeded the 50% that would be required if only those combinations occurred, as was the case for phosphatidylethanolamine (Table 6). In addition to fatty acids, other unsaturated compounds, strongly retained by the GLC stationary phase, may be esterified to the glycerol moiety in these fractions of PC.

Although the separation of rod outer segment PE, PS, and PI into molecular species was based on the separation of the PCs used as carriers, it was evident that the distribution of radioactivity varied among supraenes. Thus, while a major portion of supraenoic PC was associated with the third band, this band was only a minor component of PE (Tables 1 and 2). Most of the radioactivity in supraenoic PI was associated with the species at the origin of the plates, while half of the radioactivity from supraenoic PS appeared in the second band (Tables 3 and 4). This indicates that the proportions and fatty acid composition of supraenoic subspecies differed among phospholipids. Serine glycerophos-

TABLE 2. Molecular species of bovine retina ethanolamine glycerophospholipids

Species	Whole Retina (3)	Microsomes (6)	ROS (5)
		mol %	
Supraenes			
I	2.7	4.5 ± 0.1	6.5 ± 0.7
II	2.4	3.2 ± 0.8	2.9 ± 0.3
III	2.3	2.4 ± 0.6	2.6 ± 0.3
IV	2.4	2.2 ± 0.5	2.2 ± 0.1
V	7.4		7.5 ± 0.8
Total	17.3 ± 0.3	11.4 ± 1.2	20.6 ± 1.4
Hexaenes	52.5 ± 2.8	50.6 ± 3.8	67.4 ± 0.6
Pentaenes			
I	3.3 ± 0.2	6.5 ± 0.7	3.3 ± 0.3
II	1.6 ± 0.3		1.5 ± 0.1
Tetraenes			
I	2.2 ± 0.7	6.5 ± 1.5	4.0 ± 0.2
II	10.8 ± 0.5	11.3 ± 2.2	
Trienes	3.1 ± 0.7	3.8 ± 0.7	0.7 ± 0.1
Dienes	1.8 ± 0.3	2.1 ± 0.4	0.4 ± 0.0
Monoenes	5.8 ± 2.5	3.6 ± 0.5	0.6 ± 0.1
Saturates	1.6 ± 0.1	1.8 ± 1.0	1.0 ± 0.1

Labeled acetyldiacylglycerols were prepared as indicated in Table 1 for PC. Unlabeled acetyldiacylglycerols from PC were added as carriers. Other details as in Table 1.

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pholipids contained a high percentage of pentaenoic fatty acids, in contrast to phosphatidylinositols (**Fig. 1**). Considering that the percentage of pentaenoic phosphatidylserines was low (Table 3), it may be predicted that the second supraenoic fraction contained 22:6 and most of the 24:5, i.e., PS with eleven double bonds may be the major dipolyunsaturated species of rod outer segment PS.

Hexaenes, containing mainly docosahexaenoate and saturated fatty acids (Tables 5 and 6), constituted two-thirds of rod outer segment PE (Table 2). High percentages (more than 30%) of hexaenes were also present in ROS PC and PS (Tables 2 and 3). Hexaenes were the most abundant species in microsomal PE and PS.

Tetraenes were the major molecular species of bovine retinal PI, as is the case in other vertebrate tissues (18, 19). The level of trienes was also higher in PI than in other phospholipids of both membranes. Tetraenes represented about 70% and 50% of PI from microsomes and rod outer segments, respectively. The proportion of tetraenes in all four glycerophospholipids was higher in microsomes than in ROS.

Monoenes contributed very little to rod outer segment phospholipids. In contrast, monoenes were the major species in microsomal PC, and the second most common in PS.

Disaturated species, made up largely of palmitic acid, were also important components of microsomal PC. It is evident that saturated species were provided almost exclusively by phosphatidylcholine, inasmuch as PC was the major phospholipid in rod outer segments.

TABLE 3. Molecular species of bovine retina phosphatidylserine

Species	Whole Retina (4)	Microsomes (3)	ROS (6)
		mol %	
Supraenes			
I	3.1 ± 0.9	8.8 ± 3.5	11.8 ± 2.3
II	8.6 ± 1.6	4.9 ± 2.0	26.4 ± 4.0
III	3.7 ± 2.0		12.9 ± 1.1
Total	15.3 ± 2.5	13.9 ± 2	51.5 ± 5.0
Hexaenes	46.7 ± 3.7	42.7 ± 0.9	36.9 ± 1.1
Pentaenes			
I	5.8 ± 0.9	5.9 ± 1.1	2.9 ± 0.8
II	1.5 ± 0.2	2.7 ± 0.2	1.3 ± 0.4
Tetraenes	7.5 ± 0.8	7.9 ± 0.3	2.7 ± 0.6
Trienes	2.3 ± 0.3	3.5 ± 0.4	1.1 ± 0.3
Dienes	3.6 ± 0.7	5.1 ± 0.3	0.8 ± 0.2
Monoenes	13.8 ± 1.9	16.5 ± 2.3	2.1 ± 0.6
Saturates	3.0 ± 1.5	2.0 ± 0.1	0.6 ± 0.2

Diacylglycerols from retina phosphatidylserine were acetylated with [1-^{14}C]acetic anhydride, and those from microsomes and ROS were acetylated with [^{3}H]acetic anhydride. Other details as in Table 2.

TABLE 4. Molecular species of bovine retina phosphatidylinositol

Species	Whole Retina (4)	Microsomes (3)	ROS (3)
		mol %	
Supraenes			
I	1.1 ± 0.4	1.6 ± 0.3	4.8 ± 0.4
II	0.4 ± 0.1	0.5 ± 0.1	2.5 ± 0.4
III	0.4 ± 0.1	0.6 ± 0.1	2.1 ± 0.6
Total	1.5 ± 0.5	2.8 ± 0.3	9.0 ± 0.6
Hexaenes	8.3 ± 2.7	5.2 ± 0.5	11.8 ± 0.5
Pentaenes			
I	4.2 ± 0.4	3.9 ± 0.4	4.9 ± 0.5
II	1.2 ± 0.3	1.9 ± 0.3	1.9 ± 0.1
Tetraenes	74.9 ± 3.3	71.0 ± 1.4	49.6 ± 3.1
Trienes			
I	4.1 ± 0.6	5.3 ± 0.7	6.2 ± 1.0
II		1.3 ± 0.3	3.3 ± 0.1
Dienes	1.3 ± 0.1	2.5 ± 1.1	5.8 ± 1.1
Monoenes	2.9 ± 1.1	3.6 ± 0.7	4.9 ± 1.3
Saturates	2.1 ± 0.4	2.9 ± 0.4	3.5 ± 0.7

Labeled acetyldiacylglycerols were prepared as indicated for PS in Table 3.

DISCUSSION

This work shows that several dipolyunsaturated species occur in rod outer segment glycerophospholipids, including phosphatidylinositol; molecular species more unsaturated than hexaenes have not been described for this phospholipid. Supraenes plus hexaenes represented about 20% of PI molecular species, and the percent of docosahexaenoate in PI was higher in ROS than has been reported in other retinal membranes (10) (Fig. 1) or any mammalian tissue. A higher percent of 22:6 (25%) has been described only in the whole retina of a poikilotherm (1, 2), where rods are the predominant visual cells. If one assumes that no more than half the fatty acids in hexaenes are made up of docosahexaenoate, the results presented here suggest that most of the 22:6 in bovine retinal PI is concentrated in supraenoic species. The ratio of supraenes/hexaenes in PI is about 1, as high as in PC, and higher than in PE.

Based on the percentages of major phospholipids in ROS reported by Anderson, Maude, and Zimmerman (10) and the molecular species compositions reported here, it can be estimated that in every 100 moles of glycerophospholipids, about 11, 9, 8, and 0.1 moles are supraenoic phosphatidylcholines, -ethanolamines, -serines, and -inositols, respectively. Thus, nearly 30% of the phosphoglycerides in ROS are dipolyunsaturated, the first three contributing nearly equally with these species to the ROS membrane. However, hexaenes were

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TABLE 5. Fatty acids of bovine rod outer segment phosphatidylcholines

Species	Supraenes					Hexaenes	Pentaenes		Tetraenes	Trienes	Dienes	Monoenes	Saturates
	I(3)	II(4)	III(4)	IV(4)	V(4)	(3)	I(4)	II(4)	(4)	(2)	(4)	(4)	(4)
16:0					4.5 ± 0.26	14.5 ± 2.2	15.3 ± 1.5	15.3 ± 0.7	20.1 ± 1.3	19.4	28.4 ± 1.3	31.0 ± 3.2	85.7 ± 2.5
16:1					3.6 ± 0.3	0.5 ± 0.1		1.8 ± 0.8	1.2 ± 0.1	1.7	4.0 ± 0.3	8.7 ± 3.2	
17:0						0.5 ± 0.1				0.9	0.7 ± 0.0	0.8 ± 0.2	1.2 ± 0.1
18:0					6.4 ± 0.8	30.9 ± 1.9	30.2 ± 1.8	22.0 ± 0.6	26.0 ± 1.6	22.8	13.7 ± 0.6	11.5 ± 2.5	12.0 ± 1.8
18:1					17.4 ± 1.8	1.2 ± 0.4	1.4 ± 0.5	9.1 ± 0.8	3.1 ± 1.5	4.8	9.6 ± 3.8	47.4 ± 3.0	
18:2				2.6 ± 0.6	1.1 ± 0.5		0.3 ± 0.2	1.2 ± 0.3	0.3 ± 0.0	1.3	37.7 ± 1.3		
18:3(n-6)[a]			0.4 ± 0.1						0.3 ± 0.1	8.6			0.1 ± 0.0
18:3(n-3)[a] + 20:1									0.5 ± 0.2	1.3	3.5 ± 0.3	1.0 ± 0.6	
20:3(n-9)[a]								2.2 ± 0.3	0.7 ± 0.2	3.7			
20:3(n-6)		0.4 ± 0.2	0.2 ± 0.1	1.9 ± 0.2						19.0			
20:3(n-3) + 20:4(n-6)		0.2 ± 0.1	2.5 ± 1.0	4.7 ± 0.5	11.3 ± 0.5	0.5	1.4 ± 0.5	12.7 ± 3.3	45.0 ± 0.6	14.2			
20:4(n-3)[a]				0.4 ± 0.2				2.9 ± 0.3		1.2			
20:5(n-3)		0.2 ± 0.1	0.2 ± 0.0	0.7 ± 0.2	0.6 ± 0.2		7.3 ± 0.1						
Unidentified		0.2 ± 0.1		0.5 ± 0.2									
22:4(n-6)		0.1 ± 0.0	0.9 ± 0.3	0.4 ± 0.2	0.7 ± 0.4			6.3 ± 0.9	3.0 ± 0.2				
22:5(n-6)		1.3 ± 0.2	0.7 ± 0.5	1.9 ± 0.2			0.3 ± 0.1	22.3 ± 1.6					
22:5(n-3)	0.2 ± 0.0	2.7 ± 0.2	1.5 ± 0.1	2.8 ± 0.7	1.3 ± 0.2	0.1 ± 0.7	32.8 ± 1.9						
22:6(n-3)	95.4 ± 0.7	79.8 ± 1.5	85.1 ± 2.5	82.2 ± 4.5	45.1 ± 2.0	51.5 ± 4.8							
24:4			6.7 ± 3.0	2.1				4.6 ± 0.6	1.1 ± 0.1				
Unidentified		0.8 ± 0.3	0.3 ± 0.1	1.3									
24:5		10.5 ± 0.5	0.4 ± 0.1	1.6 ± 0.2			11.0 ± 1.0	2.5					
24:6	5.8 ± 1.1	0.9 ± 0.4	1.0 ± 0.1	1.3	8.5 ± 0.3	0.2							
26:x		3.1 ± 1.1	0.7 ± 1.0										

Acetyldiglycerides were obtained from rod outer segment PC and were resolved by argentation TLC. Methyl esters were prepared and analyzed by gas–liquid chromatography. Results are mean values ± SD from four samples.

[a] Tentative identification.

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provided mainly by phosphatidylethanolamine; 30, 13, 6, and 0.2% of the phosphoglycerides were hexaenoic PE, PC, PS, and PI, respectively, all of which accounted for nearly half the mass of the ROS phospholipids. The same calculations carried out in microsomes indicate that only 8% of the phosphoglycerides are supraenoic and 25% are hexaenoic.

The moles percent of species containing unsaturated-unsaturated fatty acid combinations in major phospholipids of ROS surpassed those predicted by Miljanich et al. (3). On the basis of fatty acid compositions of subfractions separated by TLC, these authors estimated that *at least* 24%, 24%, and 40%, respectively, of the PC, PE, and PS from ROS should be composed of species containing two unsaturated fatty acids, predominantly dipolyunsaturated species. Our results show that 31%, 21%, and 52%, respectively, were supraenoic species. However, unsaturated-unsaturated combinations of fatty acids also occurred in other molecular species, (e.g., pentaenes, tetraenes), as suggested by their separation into subspecies.

The molecular species composition of phospholipids from whole retina reflects the contribution of rod outer segments and endoplasmic reticulum, but also includes phospholipids from other retinal membranes. In general, there were larger amounts of docosahexaenoate-containing species in whole retina than in microsomes. Because microsomes were isolated from whole tissue, they represented endoplasmic reticulum from a heterogenous population of retinal cells, including the "neural" retina and the inner segments of photoreceptors. It has been demonstrated that in the base of the visual cells, an active biosynthesis of phospholipids supplies the continuous renewal of disk membranes that takes place in the apical portion of the outer segments (20, 21). As supraenes are highly concentrated in ROS and have not been detected in neural tissue, it is possible that at least part of the supraenoic species found in retinal microsomes may reflect the contribution of the endoplasmic reticulum of visual cells.

In whole retinas and in microsomes isolated from retinas incubated with various precursors, a high proportion of the radioactivity incorporated into PC, PE, PI, and PS appeared in supraenes and hexaenes.[2] High specific activities were suggested by the ratios between percent distribution of radioactivity and the percent composition shown in Tables 1–4. An outstanding example is phosphatidylinositol. At early incubation times (5–10 min), more than half the [^{3}H]-labeled glycerol or inositol accumulated in these species, which amounted

[2] Aveldaño, M. I., S. Pasquare, and N. G. Bazán. Unpublished results.

TABLE 6. Fatty acids of bovine rod outer segment phosphatidylethanolamines

Species	Supraenes			Hexaenes	Pentaenes		Tetraenes		Trienes	Dienes	Monoenes	Saturates
	I(3)	II(4)	III(4)	(4)	I(4)	II(2)	I(2)	II(4)	(2)	(2)	(2)	(2)
16:0				11.5 ± 0.8	10.4 ± 3.2	3.1	7.1	6.6 ± 1.4	24.0	23.0	24.0	67.6
16:1				0.7 ± 0.1	1.8 ± 0.7	0.7	1.1	0.6 ± 0.3	8.3	7.4	6.6	
17:0				0.5 ± 0.1				1.4 ± 0.4	0.7			
18:0				25.4 ± 1.6	30.6 ± 4.0	37.1	30.8	34.9 ± 1.2	24.0	22.1	25.4	36.8
18:1				4.7 ± 0.3	2.4 ± 1.0	3.8	13.1	4.1 ± 0.3	19.3	31.8	42.1	
18:2			0.6 ± 0.3	0.6 ± 0.2	0.3 ± 2.0			1.2 ± 0.3	3.9	16.0		
18:3(n-6)[a]		0.9 ± 0.7	5.4 ± 0.5									
18:3(n-3)[a] + 20:4			1.1 ± 0.1					0.9 ± 0.2				
20:3(n-6)		0.3 ± 0.2	25.7 ± 2.1						4.3			
20:3(n-3) + 20:4(n-6)	0.5 ± 0.2	22.2 ± 0.9	5.1 ± 0.6			5.3	39.2	44.1 ± 1.9	11.6			
20:4(n-3)[a]	0.9 ± 0.1	2.0 ± 0.4										
20:5(n-3)	0.5 ± 0.2				3.9 ± 1.1	2.0						
Unidentified			1.8 ± 0.6									
22:4(n-6)		10.0 ± 0.2	5.2 ± 3.4		1.4 ± 1.4	4.1	25.0	5.4 ± 1.5				
22:5(n-6)	1.0 ± 0.0	2.4 ± 0.2			2.8 ± 2.1	33.6	7.7					
22:5(n-6)	5.2 ± 0.2	1.4 ± 0.2	1.3 ± 0.1	0.1 ± 0.1	33.7 ± 3.1	6.9	1.0					
22:6(n-3)	82.9 ± 1.0	49.2 ± 1.2	50.4 ± 3.4	56.1 ± 0.7								
24:4		6.6 ± 0.6	2.8		6.0 ± 1.0	3.2	3.0	0.6 ± 0.2				
Unidentified	0.3 ± 0.1	1.0 ± 0.6	1.2					0.8 ± 0.1				
24:5	5.9 ± 0.6	4.1 ± 1.9	0.9		4.6 ± 1.0							
24:6	2.8 ± 0.3			0.3 ± 0.0								

Results are presented as in Table 5.
[a] Tentative identification.

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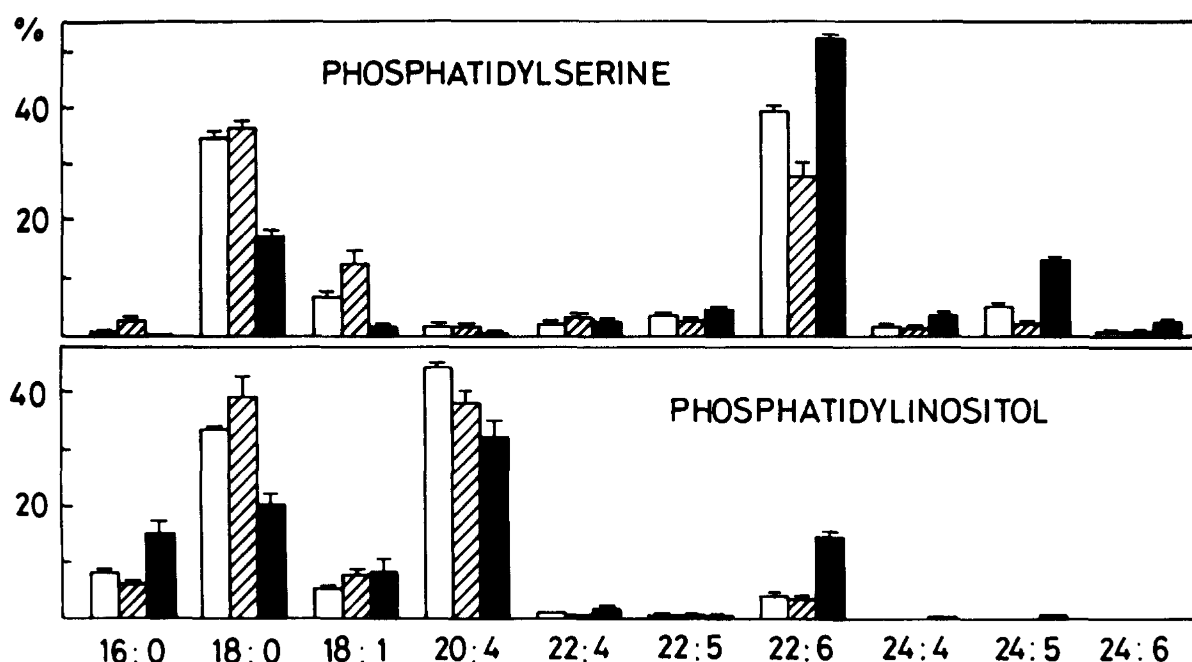

Fig. 1. Fatty acid composition of phosphatidylserine and phosphatidylinositol from whole retina (□), microsomes (▨), and rod outer segments (■). The phospholipids were methanolyzed and analyzed by GLC.

to only 10% of the PI in whole retina and microsomes (Table 4). This is consistent with the hypothesis that these species are synthesized de novo in the endoplasmic reticulum; this idea is supported by the finding of high levels of docosahexaenoate in phosphatidic acid from retinal microsomes (22, 23).

The physical properties of phospholipids depend strongly on the degree of unsaturation of their constituent fatty acids. Highly unsaturated glycerophospholipids must play a specific role in structure–function relationships in the photoreceptor membrane. A fluid membrane bilayer may be required for the rotational motion and lateral diffusion of rhodopsin (24, 25). The involvement of individual molecular species of glycerophospholipids in separate biophysical and/or biochemical functions is suggested by the fact that whole spectra from dipolyunsaturated to fully saturated species coexist in membranes as separate entities and in specific proportions for every phospholipid. It has been proposed that dipolyunsaturated species of phosphatidylserine are involved in the binding of calcium ions during visual excitation (26).

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