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Phospholipid fatty acyl spatial distribution in bovine rod outer segment disk membranes

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Abstract

The distribution of fatty acids within the phospholipid headgroup classes was investigated as a function of the age/spatial distribution of bovine rod outer segment disk membranes. The disks were separated into subpopulations based upon the cholesterol content in their membranes. Because disk membrane cholesterol content decreases as the disks are apically displaced in the rod outer segment, this separation yields disk subpopulations of different ages and from age-dependent spatial locations within the outer segment. The phospholipids, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI), of each of these subpopulations were separated and the fatty acid composition of each was determined. These data indicated that while most of the fatty acids show little or no change with age/spatial location, some pronounced changes can be observed in certain classes. Within the PC class, 16:0 dramatically decreases with disk age while the 22:6 increases with disk age. While the PE class exhibits some fatty acid changes, they are small. The PS class exhibits no significant changes in fatty acid composition. The PI class which constitutes less than 2% of the total phospholipid exhibits age-related changes in each of the fatty acids which could be measured. Most notable of these is an increase in 20:4 as the disks are apically displaced. These changes indicate a remodeling of the disk membranes which may be related to the phototransduction process or to preparation for eventual disk phagocytosis. © 1998 Elsevier Science B.V.

Keywords: Rod outer segment disk; Fatty acid; Phospholipid

1. Introduction

Rod cells are responsible for vision under conditions of low light levels. The rod outer segment (ROS) consists of a stack of flattened disks surrounded by a plasma membrane. These disks are the site of the initial events of visual transduction. Visual transduction is initiated by a conformational change in the visual pigment, rhodopsin, which is imbedded in the disk membrane. This conformational change is triggered by the absorption of light by the rhodopsin

Abbreviations: ROS, rod outer segment; GC, gas chromatography; HPLC, high-pressure liquid chromatography; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositiol

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chromophore, 11-*cis* retinal. Several spectrally defined conformational states have been identified. However, the transition from metarhodopsin I to metarhodopsin II corresponds to the activation of rhodopsin and the initiation of the cGMP cascade.

While the rod cell does not divide, the outer segment is constantly renewed. New disks are formed from evaginations of the plasma membrane at the base of the ROS and displaced toward the apical tip of the outer segment as additional disks are formed. Disks at the apical tip of the rod are shed and phagocytosed by the overlying pigment epithelium. Upon disk formation from the plasma membrane, the lipid and protein components must be sorted to achieve a new independent membrane with protein and lipid compositions distinct from that of the original plasma membrane [1–4].

The visual pigment, rhodopsin, has been extensively studied with respect to the effect of the lipid environment on its functional properties. Studies of this integral membrane protein incorporated into lipid vesicles have shown it to be sensitive to cholesterol levels [5], unsaturation of the fatty acyl chains [6–8] and phospholipid headgroup composition [8]. It is therefore important to understand the native membrane lipid environment in which rhodopsin must function. Membrane cholesterol decreases as the disks are apically displaced [9]. Physiological levels of membrane cholesterol have been shown to affect the stability of rhodopsin in disks [10]. Furthermore, ROS plasma membrane cholesterol has been implicated in inhibition of rhodopsin activation in this membrane [11].

During the lifetime of the disks the membrane phospholipids undergo turnover [12]. Therefore, there is clear potential for remodeling of the disk membranes during their transit from the rod base to the apical tip. Furthermore, disk formation is inhibited in the absence of dietary linoleic or linolenic fatty acids [13]. Therefore, unsaturated fatty acids are essential for the disk membrane biogenesis. It was shown previously, in this laboratory, that there are no significant changes in the total phospholipid composition of disks as they are apically displaced [4]. However, there remained the possibility that the fatty acid composition within the phospholipid headgroup classes could change as the disks are apically displaced. In this study it is demonstrated that, in some cases, the fatty acyl composition of the disk membrane phospholipids is remodeled as a function of disk age and spatial location.

2. Materials and methods

2.1. Preparation and separation of disk subpopulations

Disks were prepared from frozen bovine retinas (J. Lawson, NE) by ficoll floatation [14]. The isolated disks were treated with digitonin and separated into various subpopulations as previously described [15]. The cholesterol, phospholipid and protein content of the isolated subpopulations was determined as described below. All manipulations of the rod outer segment, disk and plasma membranes were performed under a Kodak 1A red filter. The buffers used were made 1 mM in EDTA and perfused with nitrogen or argon to reduce lipid oxidation [16].

2.2. Determination of phospholipid composition

ROS disks were extracted as described in [17]. The lipids were isolated from the lower organic phase, dried under nitrogen and then resuspended in hexane. The phospholipid headgroup composition of these total lipid extracts was immediately determined using high-pressure liquid chromatography (HPLC) on a LiChrosorb SI-60 (silica gel column). Column elution was carried out with a gradient of hexane : propanol : water as previously described [18] at a flow rate of 1 ml/min at room temperature. The individual lipid components were monitored directly by UV absorption at 206 nm. The phospholipids were identified by comparison to known standards (Avanti Polar Lipids). The collected fractions were then analyzed by gas chromatography to determine the fatty acid composition of each headgroup.

2.3. Determination of fatty acid composition

The fractions corresponding to each of the phospholipids, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI), were dried under nitrogen. These fractions were subjected to mild alkaline

methanolysis and the resulting fatty acid methyl esters were analyzed by gas chromatography as previously described [15]. The initial temperature of the column was 160°C and increased at the rate of 6°C/min to a final temperature of 260°C. Both Varian, Model 3700, equipped with a flame ionization detector and Varian 4270 integrator and Chrompack–Packard equipped with a Spectra Physics integrator were used with equally good results. Peaks were identified by comparison to reference methyl ester mixtures, PUFA no. 189-1, 189-3 and 189-15 purchased from Sigma.

Table 1
Fatty acid composition of the phospholipid classes averaged among all the disk subpopulations

	PC	PE	PS	PI
16:0	26.5	17.0	2.8	14.14
18:0	17.4	27.4	27.0	37.7
18:1	8.4	5.0	5.0	
20:4	6.5	7.1	2.7	41.2
22:4	3.1	2.5	6.7	
22:5	8.5	10.0	11.9	
22:6	34.5	35.1	40.3	8.9

2.4. Additional assays

Phosphate was determined by the method of Bartlett [19] as modified by Litman [20]. Cholesterol was determined as described by Allain [21]. Protein was determined as described by Lowry [22].

3. Results and discussion

ROS disk membranes were separated into subpopulations which vary in membrane cholesterol to phospholipid mole ratio by exploiting the density change induced by the interaction of the detergent digitonin with membrane cholesterol [15]. As the disks are apically displayed, the cholesterol to phospholipid mole ratio of the membranes progressively decreases. Thus, the cholesterol to phospholipid ratio reflects the age/spatial distribution of disks in the outer segment [9].

The phospholipid headgroup composition of each disk membrane subpopulation was determined in an earlier study [3]. In that work it was shown that PE and PC made up approximately 45 and 43%, respectively, of the total phospholipid in each of the disk subpopulations. PS and PI content of the disks was approximately 12 and 1–2%, respectively. These values are in good agreement with previous determinations of the phospholipid composition of the total disks [23] and indicate that the overall phospholipid headgroup composition of the disks does not change as the disks are apically displaced.

In the present study, the major phospholipids, PC, PE, PS and PI, were isolated from the subpopulations of disks which exhibited different membrane cholesterol content. The phospholipids were separated using HPLC. The phospholipids represented by each HPLC peak were then further analyzed by GC to determine the fatty acid composition of each of the phospholipids. Although disaturated and dipolyunsaturated phospholipids have been reported in disk membranes [24], the techniques used here do not allow this determination. The average overall fatty acid composition for each of the phospholipid classes is presented in Table 1. These values are in good agreement with previously published fatty acid distributions of the fatty acids amongst the phospholipid classes [25].

In Figs. 1–4 the distribution of fatty acids within each headgroup class is presented as a function of the cholesterol to phospholipid ratio with each point representing a single determination. As noted above, the cholesterol to phospholipid ratio corresponds to the disk age/spatial distribution; high cholesterol levels correspond to newer, basal disks. Although additional fatty acids were identified, only the fatty acids which constituted greater than 1–2% in each of the classes are presented in these figures. These data suggest that the fatty acid composition, in some cases, changes with disk age. Therefore, the data were further analyzed to determine the statistical significance of the changes in the fatty acid composition. A slope of the line best fitting the data which is significantly different from zero indicates a change in the fatty acid composition with respect to the disk cholesterol composition. The number of determinations which defined the relationship as well as P values for each of the fatty acids determined is given in Table 2.

Within the PC headgroup class 16:0 decreases with disk age and 22:6 increases with disk age.

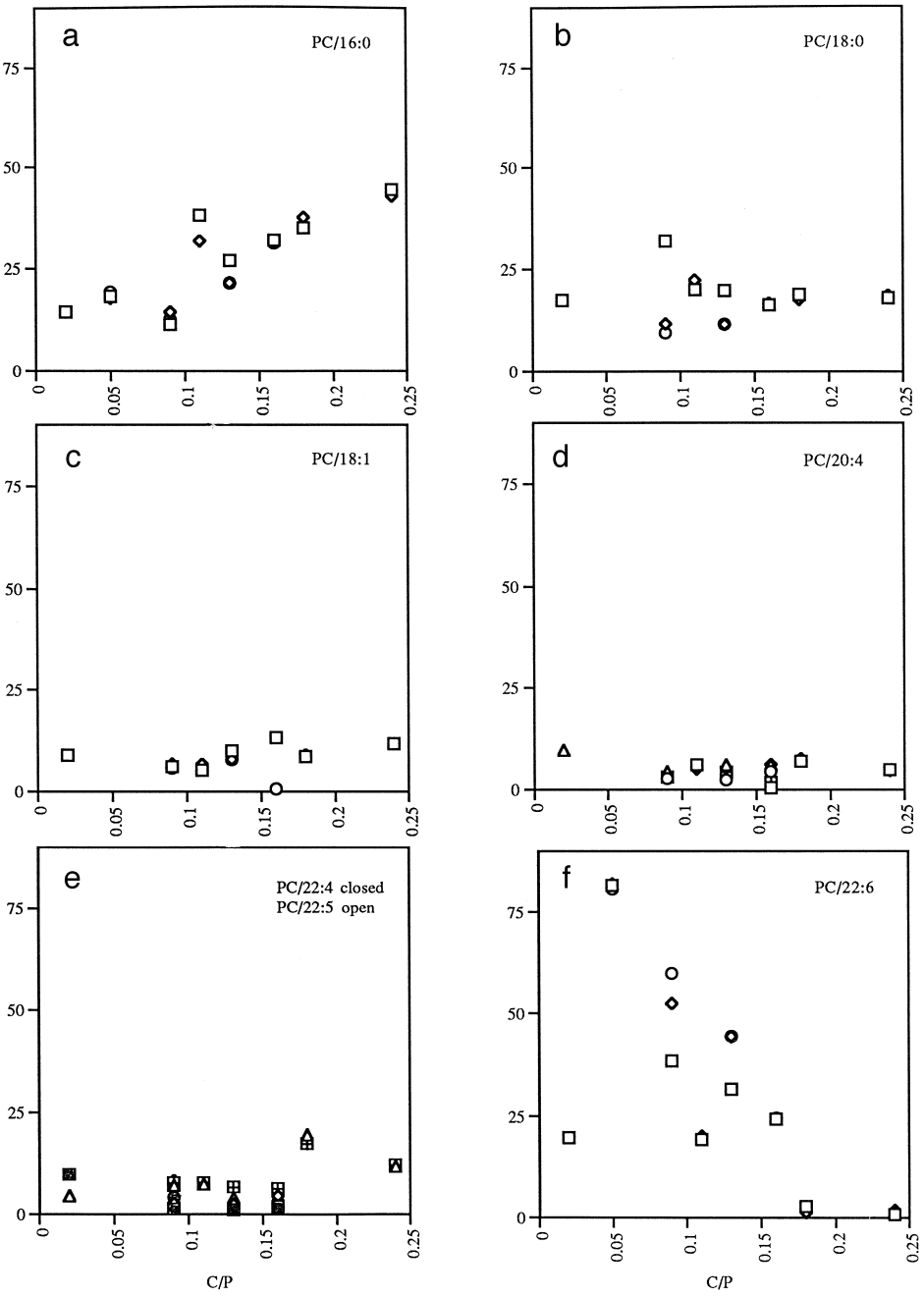

Fig. 1. Fatty acid composition within the phosphatidylcholine (PC) headgroup class as a function of the cholesterol to phospholipid ratio of the disks from which they were isolated. Fatty acids are presented as a percentage of the total fatty acids within the phospholipid class. Each point represents an individual determination and the different symbols represent independent experiments. The fatty acids shown are (a) 16:0, (b) 18:0, (c) 18:1, (d) 20:4, (e) 22:4/22:5 and (f) 22:6.

While 22:4/22:5 also show age-related changes, the confidence levels and the magnitude of the changes in these two fatty acids are much less than for 16:0 and 22:6. The increase of 22:6 and decrease of 16:0 is also intriguing in relationship to other observations. First, highly unsaturated membranes provide an unfavorable environment for cholesterol. Therefore, this change is consistent with

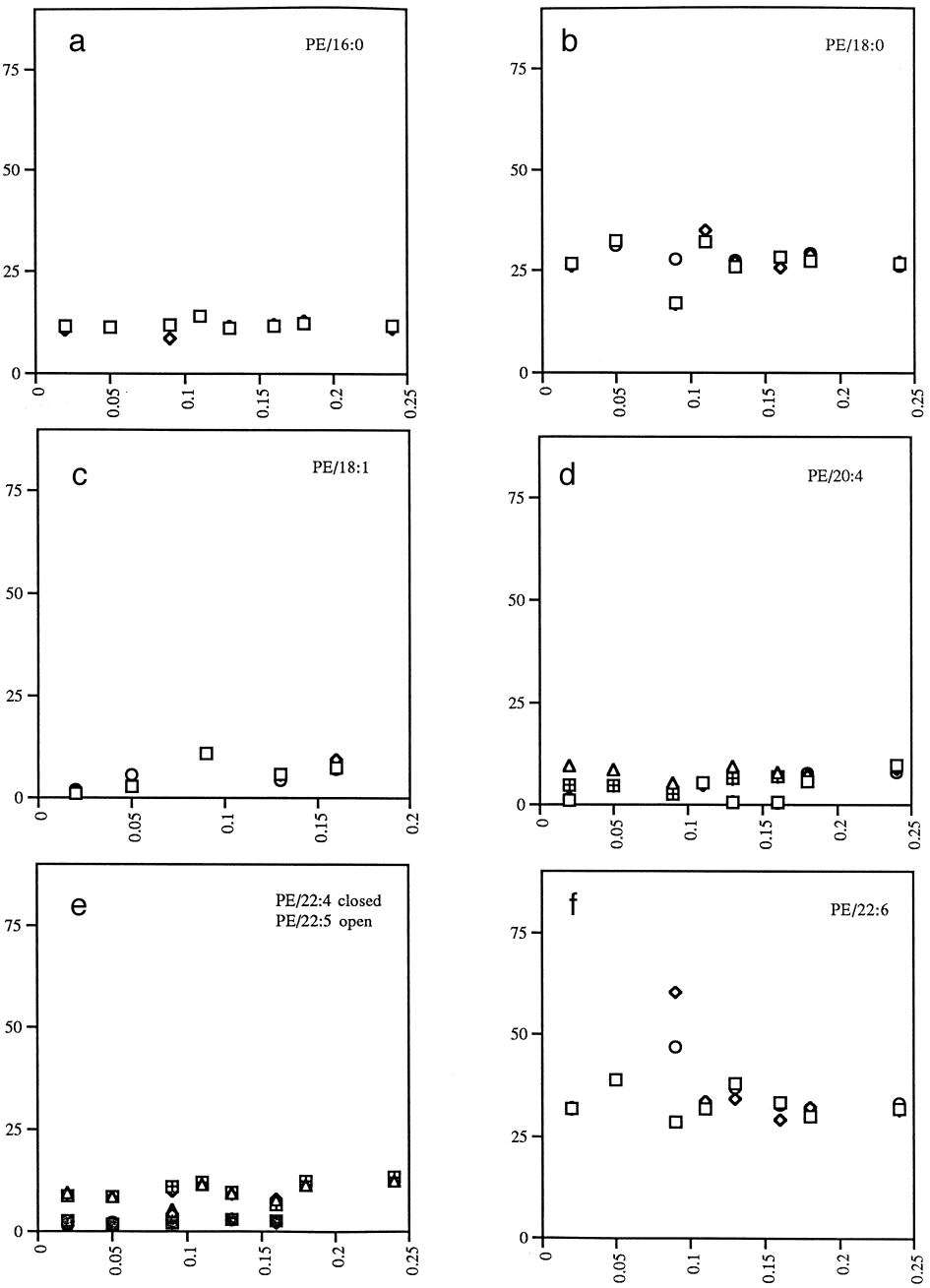

Fig. 2. Fatty acid composition within the phosphatidylethanolamine (PE) headgroup class as a function of the cholesterol to phospholipid ratio of the disks from which they were isolated. Fatty acids are presented as a percentage of the total fatty acids within the phospholipid class. Each point represents an individual determination and the different symbols represent independent experiments. The fatty acids shown are (a) 16 : 0, (b) 18 : 0, (c) 18 : 1, (d) 20 : 4, (e) 22 : 4/22 : 5 and (f) 22 : 6.

our earlier observation that cholesterol decreases with age/spatial displacement of the disks. Secondly, it has been shown that both cholesterol and polyunsaturated phospholipids can modulate the metarhodopsin I/metarhodopsin II conformational equilibrium in favor of metarhodopsin II [5,6]. Therefore, these data suggest that rhodopsin in the apical disks may be more readily activated than rhodopsin in basal disks.

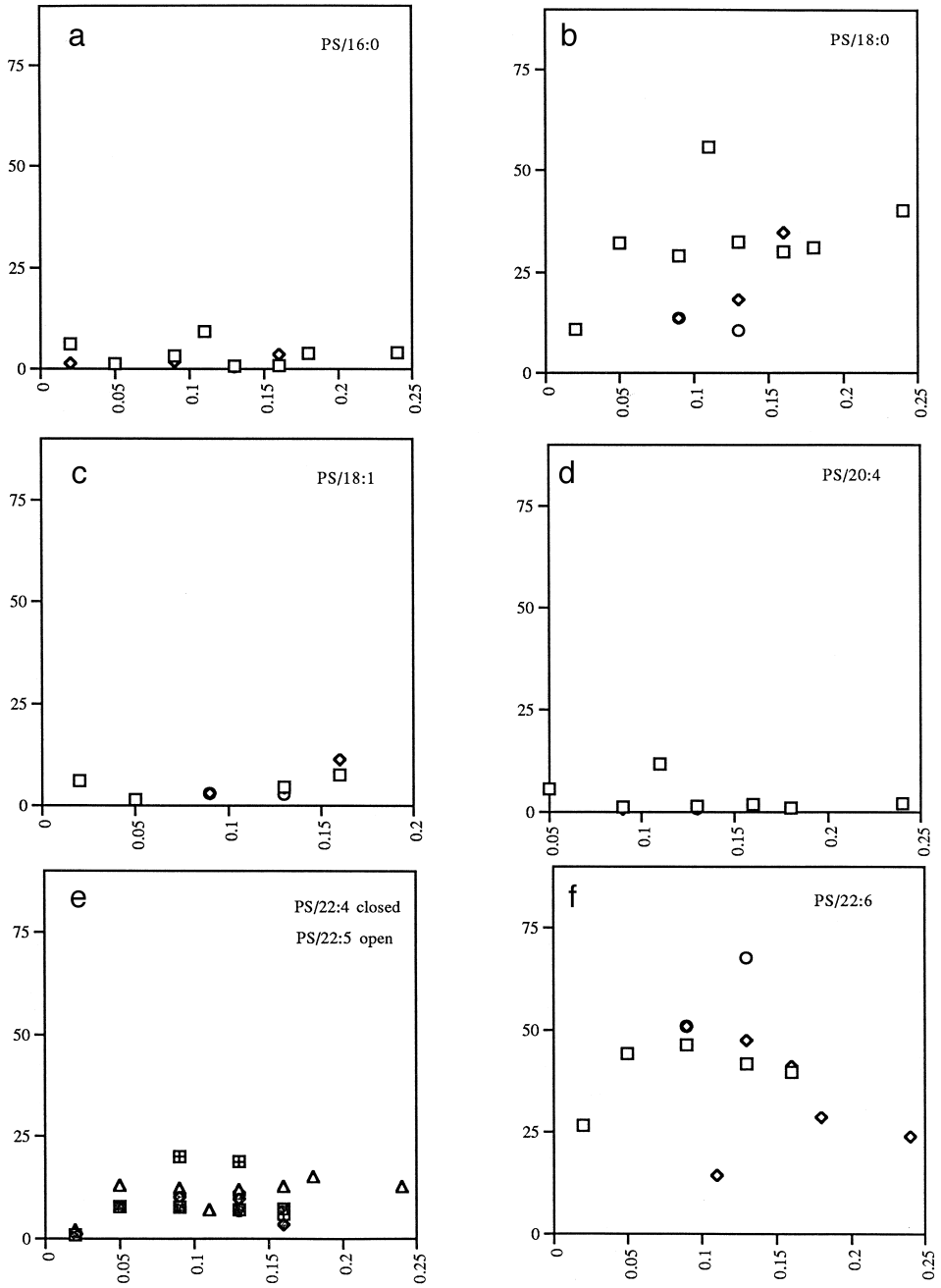

Fig. 3. Fatty acid composition within the phosphatidylserine (PS) headgroup class as a function of the cholesterol to phospholipid ratio of the disks from which they were isolated. Fatty acids are presented as a percentage of the total fatty acids within the phospholipid class. Each point represents an individual determination and the different symbols represent independent experiments. The fatty acids shown are (a) 16:0, (b) 18:0, (c) 18:1, (d) 20:4, (e) 22:4/22:5 and (f) 22:6.

Within the PE class, the data from three of the fatty acids suggest an age-related change, although these changes are quite small compared to those observed for PC. A possible reason for the lack of change in 22:6 within the PE class may be found in recent studies on phospholipids containing 22:6 [26]. It was observed that when 22:6 was present in PE, this lipid could dramatically lower the bilayer to

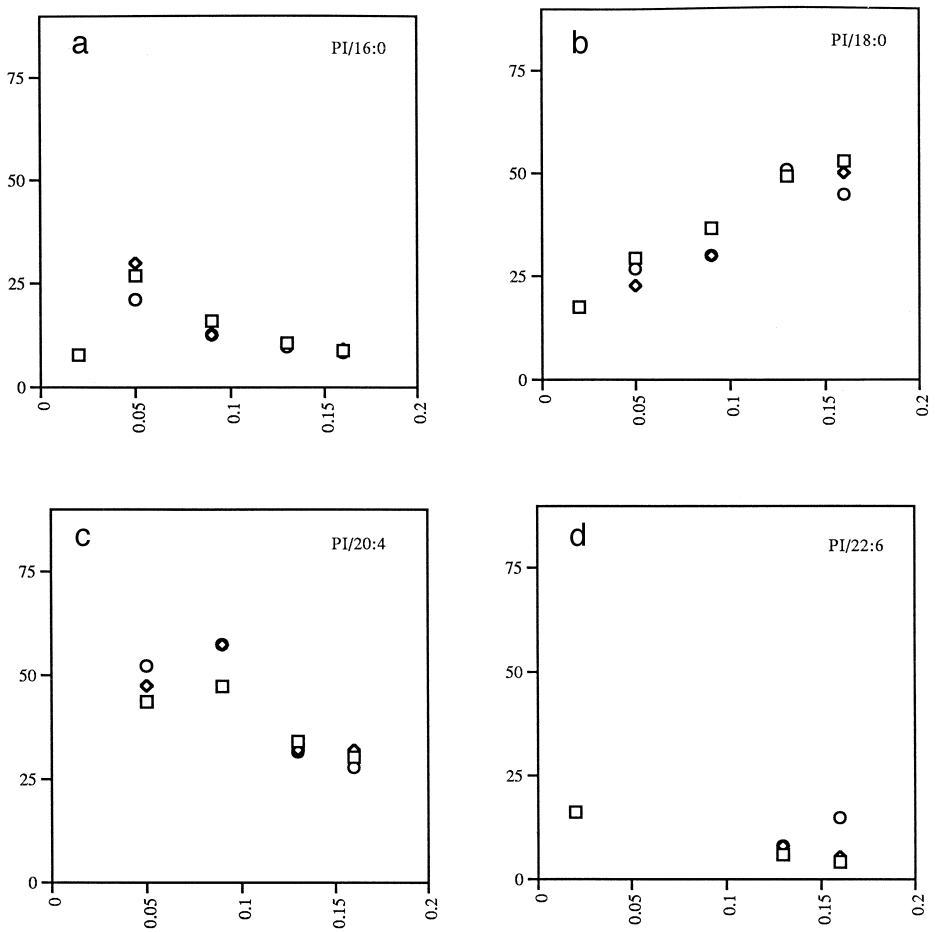

Fig. 4. Fatty acid composition within the phosphatidylinositol (PI) headgroup class as a function of the cholesterol to phospholipid ratio of the disks from which they were isolated. Fatty acids are presented as a percentage of the total fatty acids within the phospholipid class. Each point represents an individual determination and the different symbols represent independent experiments. The fatty acids shown are (a) 16:0, (b) 18:0, (c) 20:4, (d) 22:6.

hexagonal phase transition temperature. However, when 22:6 was present in PC, the opposite was true. This suggests that 22:6, when part of PE may, destabilize the bilayer. It was also shown that 22:6 in PE can increase the activity of protein kinase C (PKC) by enhancing the partitioning of the enzyme to the membrane. In PC, 22:6 does not have this effect. Therefore, by only changing 22:6 on PC the outer segment can potentially shift the metarhodopsin I/metarhodopsin II conformational equilibrium in apical disks relative to basal disks without altering the membrane stability or possibly associated enzyme activity.

PI is present only at 1–2% of the total disk phospholipid. Therefore, reliable data for only four of the fatty acids could be determined. Of these fatty acids, significant changes were particularly evident in the 18:0 and 20:4 species. The 18:0 decreases with age while the later increases. These changes may be related to the rapid turnover exhibited by PI relative to the other phospholipids of the disk membrane. Interestingly, 22:6 does not exhibit a significant change. It is also interesting to note that arachidonic acid (20:4) is one of the fatty acids which exhibits a statistically significant age-related change and that this relationship is most significant within the PI phospholipid class. As arachidonic acid is a precursor of prostaglandins, this is especially intriguing in that it was recently found that prostaglandin production is linked to disk phagocytosis and apoptosis [27,28].

Table 2
Stastical significance of changes in fatty acid composition with respect to age/spatial location of the disk

Headgroup class	Fatty acid	Number of determinations	95% confidence	*P* value
PC	16:0	19	Yes	< 0.00001
	18:0	16	No	0.976
	18:1	16	No	0.116
	18:2	11	No	0.769
	20:4	16	No	0.59
	22:4	10	Yes	0.026
	22:5	16	Yes	0.038
	22:6	19	Yes	0.0002
PE	16:0	22	No	0.857
	18:0	23	No	0.871
	18:1	13	Yes	0.005
	20:4	21	Yes	0.02
	22:4	15	No	0.140
	22:5	23	Yes	0.0066
	22:6	23	No	0.223
PS	16:0	13	No	0.973
	18:0	13	No	0.164
	18:1	9	No	0.188
	20:4	10	No	0.447
	22:4	9	No	0.477
	22:5	12	No	0.410
	22:6	13	No	0.545
PI	16:0	13	Yes	0.0274
	18:0	13	Yes	< 0.00001
	20:4	12	Yes	0.0023
	22:6	7	No	0.1356

Finally, the fatty acids within the PS class show no significant changes during the lifetime of the disk.

The role which the lipid bilayer plays, beyond that of permeability barrier in membrane function, is complex and is not completely understood. Cellular membranes are composed of arrays of phospholipids with different headgroup and fatty acyl components as well as various sterols and other neutral lipids. However, individual membranes exhibit a complex lipid composition characteristic of the particular membrane to maintain optimal cell function. The data presented here indicate that the lipids in the disk membranes can be specifically altered during its lifetime. While in most cases the fatty acid composition within headgroup classes remains constant, there are some clear exceptions. The changes in these lipids indicate a remodeling of the fatty acyl composition of the disk membrane as the disk is apically displaced. This remodeling of the membrane may affect the functioning of the disk in visual transduction and/or it may prepare the disk for eventual shedding and phagocytosis.

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