Detailed Characterization of the Lipid Composition of Detergent-Resistant Membranes from Photoreceptor Rod Outer Segment Membranes

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PURPOSE. In recent years, detergent-resistant membranes (DRMs) have been isolated in vitro models of lipid rafts, from photoreceptor outer segments (ROS), and the localization of a specific complement of photoreceptor proteins has been demonstrated. However, surprisingly little is known about the lipid composition of these important membrane domains. The present study provides the first characterization of phospholipids and fatty acids from ROS-derived DRMs.

METHODS. Bovine ROS membranes were incubated with 1% Triton X-100 at 4°C and subjected to density gradient centrifugation to isolate DRMs from the parent membranes. Lipids of ROS and DRMs were separated by two-dimensional, thin-layer chromatography and converted to methyl esters, and fatty acids were analyzed by gas chromatography. Proteins of ROS and DRMs were analyzed by SDS-PAGE and Western blot analysis.

RESULTS. The DRMs represented 8% and 3%, respectively, of total ROS lipid and protein. In general, DRMs were enriched in saturated fatty acids when compared with ROS membranes. Relative to ROS, DRMs were enriched in free fatty acids (FFAs) and a specific phosphatidylcholine (PC) fraction that was almost devoid of polyunsaturated fatty acids (PUFAs). DRMs contained less phosphatidylethanolamine (PE) and phosphatidylserine (PS). Ceramide (CM) from ROS contained PUFAs but no saturated fatty acids; the converse was true of CM from DRMs. Docosahexaenoic acid was diminished in DRMs and was not detected in the FFAs, but was equally abundant in ROS and DRM PE. ROS-derived DRMs were dramatically enriched in caveolin-1, contained significant amounts of transducin-α and c-Src, and were relatively devoid of arrestin.

CONCLUSIONS. The relatively saturated lipid environment observed in DRMs is likely to promote the localization of signaling proteins modified with saturated fatty acyl chains. Based on the lipid composition of DRMs, the authors conclude that they would not efficiently support phototransduction. (Invest Ophthal Vis Sci. 2005;46:1147–1154) DOI:10.1167/iovs.04-1207

Klausner et al.1 were among the first to appreciate the significance of lipid microdomains within the cell membrane. One type of membrane domain is characteristically insoluble in cold Triton X-100 and can be isolated by flotation on sucrose density gradients. These preparations are perhaps more appropriately called detergent-resistant membranes (DRMs), but Brown and Rose2 were the first to call them “lipid rafts.” The DRMs are thought to represent a reasonable in vitro approximation of an endogenous membrane microdomain.3 A growing body of evidence suggests that the decreased fluidity of these microdomains (relative to the surrounding membrane) is attributable to their enrichment in cholesterol, sphingolipids, and lipids containing saturated fatty acyl chains.4–6 Many significant biological activities are ascribed to the DRM microdomain, but there is still much debate regarding their relevance, or even existence, in living cells.7 The lipid environment of rafts tends to segregate proteins modified with saturated fatty acids, and several studies have documented the localization of acylated signaling proteins, including G-protein α subunits and nonreceptor tyrosine kinases to DRMs (reviewed in Resh8). This finding has led to the idea that lipid rafts may act as organizing centers that localize a variety of signaling molecules.9 Although the structure and function of lipid rafts have been extensively studied in a variety of cellular systems, there has been little work in photoreceptors.

Twenty-five years ago, Andrews and Cohen9 observed membrane microdomains in amphibian and rodent photoreceptors, which they termed “particle-free patches” (PFPs). These domains colocalized with the cholesterol-binding antibiotic filipin in freeze-fracture electron micrographs. Seno et al.9 first isolated DRMs from bovine photoreceptor ROS and several groups, including ours, have since localized a variety of proteins involved in visual transduction to the DRMs.9–15 We11 (and others10,15) also have demonstrated that photoreceptor DRM are enriched in caveolin-1, a protein component of specialized lipid raft domains called caveolae.

The mechanisms governing localization of phototransduction proteins to lipid rafts are presently unclear. It is possible, however, that the unique lipid environment within the raft may be involved. To date, we know only that there is a ~2.5-fold higher cholesterol-phospholipid ratio in DRMs of ROS and ROS disc membranes10,11 and that the sphingomyelin (SM) content of ROS disc-derived DRMs is ~3.5-fold higher than that in the parent membranes.10 Given the unique lipid composition of ROS membranes16 and the need to understand how photoreceptor proteins might be localized to DRM domains, it is important to assess DRM lipid composition in photoreceptors. We report herein the first detailed molecular characterization of lipids from photoreceptor DRMs. Fridkisson et al.17 Pike et al.18 and Pito et al.13 undertook similar experiments using mast cell, epidermal carcinoma cell, and neuronal cell cultures, respectively. Our findings compare favorably with theirs, in that the DRM lipids had higher degrees

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of saturation relative to the parent membranes, although there were differences between our bovine photoreceptor outer segment DRMs and those derived from cultured cells.

**METHODS**

**Materials**

Polyclonal antibody against caveolin-1 was purchased from BD Biosciences (Lexington, KY) and polyclonal antibodies against transducin-α and pan-Src from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Polyclonal antibody against arrestin was a gift from Igal Gery (National Eye Institute, Bethesda, MD). Glycine, Tween 20, sodium dodecyl sulfate (SDS), 2-mercaptoethanol, and prestained molecular weight markers were purchased from Bio-Rad (Hercules, CA); the blue gel-staining reagent (Gelcode Blue) from Pierce (Rockford, IL); and silver-staining kit and precast gels (Novex) from Invitrogen (Carlsbad, CA). All other chemicals were purchased from Fischer Scientific (Pittsburgh, PA) or Sigma-Aldrich (St. Louis, MO). Lipid standards were purchased from Nu-Chek Prep (Elysian, MN) or Sigma-Aldrich.

**ROS and DRM Preparations**

Light-adapted bovine retinas were obtained from Mikkelson Beef, Inc. (Offermanns, City, OK). Three independent retina homogenates containing 18 to 29 fresh retinas were used to generate the ROS fractions.20,21 DRMs were made directly from each of these ROS preparations, by using a modification11 of the method of Seno et al.9 To prepare ROS, retinas were homogenized in buffer A (10 mM Tris-HCl [pH 7.4], 70 mM NaCl, 2 mM MgCl₂, 0.1 mM EGTA, and protease inhibitors; Roche, Mannheim, Germany) containing 1.126 g/mL (34%) sucrose, in a Teflon glass homogenizer, and centrifuged at 500g for 10 minutes at 4°C to generate a supernatant fraction containing crude ROS. This supernatant was diluted with buffer A and centrifuged at 17,000g for 30 minutes at 4°C, and the pellet was resuspended in 1.177 g/mL (48%) sucrose in buffer A and placed at the bottom of a discontinuous sucrose gradient of 1.177 (48%), 1.145 (59%), 1.126 (34%), and 1.106 (28%) g/mL, and centrifuged at 113,000g for 90 minutes at 4°C. The band (1.106-1.126 interface) containing purified ROS was collected, diluted with buffer B (10 mM Tris-HCl [pH 7.4], 70 mM NaCl, 2 mM MgCl₂, and 0.5 mM EDTA) and pelleted by centrifugation at 27,000g for 30 minutes at 4°C. The purified ROS pellet was resuspended to a final concentration of ~5 mg/mL total protein in buffer B.

For DRM isolation, ROS membranes were solubilized in ice-cold buffer B containing 1% Triton X-100 to a final protein concentration of 3.5 mg/mL, triturated by four passes through a 20-gauge needle, and allowed to stand on ice for 10 minutes. The Triton X-100–lipid phosphorous molar ratio in these solubilized ROS lysates was ~3:1. The mixture was adjusted to 0.9 M sucrose with 2.4 M sucrose (diluted in buffer B without Triton X-100) and applied to another discontinuous sucrose gradient (0.9 M/50.8%, 0.8 M/27.4%, 0.7 M/24%, 0.6 M/20.5%, and 0.5 M/17.1%) and centrifuged (250,000g, 20 hours, 4°C) in a rotor (model SW55Ti; Beckman-Coulter, Fullerton, CA). The material at the 0.5- to 0.6-M interface was collected for protein and lipid analysis of DRMs. In some cases, equal-volume (0.5 mL) fractions were collected from sucrose gradients and analyzed by SDS-PAGE and immunoblotting as previously reported.11 Protein content of ROS and DRMs was determined by bicinechonic acid (BCA) assay (Pierce) using bovine serum albumin as a standard.

**Lipid Extraction**

Total lipids from ROS and ROS-derived DRMs were extracted in chloroform-methanol-water (1:1:1) according to the method of Bligh and Dyer.22 The aqueous phase contained diethylenetriaminepentaacetic acid (DTPA) as an iron chelator. After the initial extraction, the organic phase was collected, and the aqueous phase was extracted a second time, keeping the chloroform-methanol-water ratio at 1:1:1. The total volume of each extraction was at least 6 mL, with at least 2 mL of chloroform. The chloroform phases were combined and then extracted with Folch theoretical upper phase (chloroform-methanol-water; 3:4:8). The total lipid extracts were concentrated and stored at −80°C under N₂ in a known volume of chloroform-methanol (1:1, vol/vol).

**Thin-Layer Chromatography**

Individual lipids in the total lipid extracts were separated by HL-high performance thin-layer chromatography plates (HPTLC; Analtech, Newark, DE) and a two-dimensional, three-solvent method described previously.23–25 Lipid spots on the HPTLC plates were localized with iodine vapors for visual comparison of ROS and DRM lipids. The plates were stained with dichlorofluorescein for gas chromatographic analysis of fatty acids in individual lipid classes (described later).

**Fatty Acid Derivatization and Gas–Liquid Chromatography**

Dichlorofluorescein-stained lipid spots were scraped from the TLC plates, and esterified fatty acids were converted to methyl esters for gas-liquid chromatography (GLC). Silica from each spot or an aliquot of the lipid extract was added to a screw-top test tube and a mixture of pentadecanoic acid (15:0), heptadecanoic acid (17:0), and heneicosanoic acid (21:0) was added as an internal standard. Toluene (200 μL) and 1 mL of 2% H₂SO₄ in methanol were added. The tube was sealed under N₂ with Teflon-lined caps, sonicated, and heated at 100°C for 65 minutes. Tubes were cooled on ice, 1.2 mL of H₂O was added, and fatty acid methyl esters were extracted three times with 2.4 mL hexane, dried under N₂, and dissolved in 20 μL nonane. The fatty acid compositions were determined by injecting 3 μL of each at 250°C with the split ratio set to 20:1 using a DB-225 capillary column (30 m x 0.53 mm inner diameter; J&W Scientific, Folsom, CA) in a gas-liquid chromatograph (model 6890N; Agilent Technologies, Wilmington, DE) and an autosampler (model 7683; Agilent Technologies). The column temperature was programmed to hold at 160°C for 1 minute, then increased to 220°C at 1°C/min, and held at 220°C for 10 minutes. Helium carrier gas flowed at 4.2 mL/min. The hydrogen flame ionization detector temperature was set to 270°C. The chromatographic peaks were integrated and processed on computer (ChemStation software; Agilent Technologies). Fatty acid methyl esters were identified by comparison of their relative retention times with authentic standards, and the relative mole percentages were calculated.

**SDS-PAGE and Immunoblot Analyses**

SDS-PAGE was performed according to the method of Laemmli.26 Separated proteins were transferred to nitrocellulose membranes (0.45 μm; Immobilon P; Pharmacia Biotech, Piscataway, NJ) with electrobottlers (MiniGenie or Genie; Idea Scientific Co., Minneapolis, MN). Membranes were blocked for 1 hour at room temperature or overnight at 4°C with 5% bovine serum albumin in Tris-buffered saline (10 mM Tris-HCl [pH 7.4]) and 0.1% Tween 20 (TBST). Incubations with primary antisera were performed for 2 hours at room temperature followed by three washes with TBST. Blots were then incubated for 1 hour with horseradish peroxidase–conjugated goat anti-rabbit or goat anti-mouse IgG and washed 6× with TBST. Immunoreactions were detected using enhanced chemiluminescence substrates (ECL; Amersham Pharmacia Biotech).

**Statistics**

All data are expressed as the mean ± SD (n = 3). Multivariate analysis of variance with post hoc Newman-Keuls tests determined significant differences between ROS and DRMs (P < 0.05).

**RESULTS**

**Gross Protein and Lipid Content of DRMs**

Elliott et al.11 have shown that DRMs isolated from bovine ROS are enriched in cholesterol. To characterize the lipid profiles of
these DRMs more fully, ROS were prepared from light-adapted bovine retinas and incubated with Triton X-100. We focused our analyses on light-adapted ROS membranes because no differences in the general lipid composition (e.g., cholesterol-phospholipid ratio) of DRMs derived from dark-adapted and in vitro light-exposed bovine ROS membranes were observed in a prior study.\textsuperscript{10} Protein and lipid phosphorous assays of the ROS and DRMs demonstrated that the DRMs accounted for 2.7% of the total ROS protein and 8.3% of total ROS phospholipid, and this represented a more than threefold increase in the lipid-protein ratio compared to ROS membranes. The recovery of lipid phosphorous in the DRM fraction is approximately twofold less than that reported for DRMs from ROS disc membranes.\textsuperscript{10} We speculate that this difference is due to the way comparisons were made between DRMs and non-DRM fractions. In our study, all comparisons were made between DRMs and ROS membrane-starting material, whereas the earlier study compared the DRM fraction to a second, “red-orange” band visible on the sucrose gradient and designated as “Triton-soluble.” If 100% of the detergent-soluble lipid was not collected in this visible Triton-soluble band, then this would underestimate the percentage of detergent-soluble lipid and thus overestimate the DRM-associated lipid. Relative to the parent ROS membranes, the DRM lipid to protein ratio was approximately three times higher, consistent with the nearly twofold increase in phospholipid-protein found in rafts from epidermal carcinoma cells.\textsuperscript{10} As previously reported,\textsuperscript{11} silver-stained gels showed that the ROS-derived DRMs had a protein pattern distinct from ROS and clearly enriched in caveolin-1 (Fig. 1).

**Characterization of Lipid Classes**

HPTLC separation of total lipids from parent ROS and DRM fractions (Fig. 2) revealed striking differences between the two fractions. Three spots of phosphatidylincholine (PC) were quite distinct between ROS and DRMs. There was greater SM staining on the DRM plate. Ceramide (CM), which can be either a precursor or a product of SM metabolism,\textsuperscript{27} was not detected in the ROS.

The HPTLC plates were stained with dichlorofluorescein and the mass of each lipid spot was determined by quantitative GLC of the fatty acids in each lipid. For free fatty acid, SM, CM, and the lysolipids (LPE and LPC), one mole of fatty acid equates to one mole of lipid. There are two moles of fatty acid per mole of the other lipids. This quantitative analysis, reported in Figure 3, reaffirms the qualitative results seen in Figure 2, by showing significant differences between the DRMs and the ROS membranes from which they were derived. Relative to the parent ROS membrane, the DRM fraction had a lower PE-PC ratio (0.82 vs. 1.4) and 2.8 times more free fatty acid. PE and PS were less abundant in DRMs. Enrichment of SM and CM (though not statistically significant) was evident in the DRMs, and there was a PC spot (PC1) that was highly enriched. This spot had the lowest mobility and highest degree of saturation (discussed later). Polyunsaturated fatty acid-enriched PC isoforms (PC2 and PC3) were less abundant in DRMs. Although Figure 2 suggests there may be more phosphatidylinositol in DRMs than in ROS, this relationship could not be shown by GLC analysis of fatty acids.

**Characterization of Fatty Acids**

The fatty acid content of total lipids from ROS and DRMs are compared in Figure 4. Generally, the DRMs were enriched in 16:0 and depleted in unsaturated fatty acids (18:1n-9, 20:4n-6, 22:4n-6, 22:5n-6, and 22:6n-3). The greatest difference between ROS and DRMs was the 2.8-fold enrichment of 16:0 in DRMs over ROS and the 2.1-fold enrichment of 22:6n-3 in ROS over DRMs. Similar amounts of 18:0 were present in both fractions.

**Figure 1.** DRMs isolated from ROS membranes had a unique protein composition and were enriched in caveolin-1. Equal amounts (5 μg) of protein from DRMs and ROS parent membranes were subjected to SDS-PAGE and either silver staining (left) or immunoblot analysis with anti-caveolin-1 (right). Silver staining demonstrated differences in the protein profiles of ROS and DRMs. Immunoblot analysis of caveolin-1 revealed substantial enrichment of this protein in the DRM fraction. Corresponding molecular weight standards are on the left.

**Figure 2.** Lipids extracted from DRMs and ROS membranes were not the same. Total lipids were extracted and separated in two dimensions on HPTLC plates. Individual lipid spots were revealed with iodine vapors. HPTLC plates were loaded with 100 nanomoles lipid phosphorous. The unidentified spots in vertical alignment along the right of each plate are neutral lipid standards.
We also determined the fatty acid profiles of individual lipid classes separated by HPTLC (Fig. 5). As shown in Figure 2, PC was resolved into three spots: PC1, -2, and -3. The PC1 spot of DRMs had only trace amounts of PUFA (<2% 18:1n-9, 10% 18:0), and the remainder was 16:0 (87%). With the exception of 8% more 18:1n-9 and 4% more 18:0 (14%), the PC1 of ROS was little different from DRMs. The principal n-3 fatty acid of the retina, 22:6n-3, was the same in PE of ROS and DRMs (34%-36%), but was relatively enriched in ROS over DRMs in all other lipid classes. PS and PC3 of ROS contained 34% and 59% more 22:6n-3 than DRMs, respectively. The PC2 of ROS also appeared to have more 22:6n-3 than DRMs, but this difference was not significant.

The fatty acid composition of the different lipid classes suggests that there was segregation of specific lipids into the DRMs. This is particularly evidenced by the relative absence of 16:0 in PC2 and -3 of ROS. Also, 18:1n-9 was 12% of the total in PC3 of ROS, yet it was not detected in PC3 of DRMs. The 18:0 of PC3 was more than twice as abundant in the DRMs, whereas PC2 of ROS and DRMs contained the same relative proportion of 18:0 (~43%). Similarly, 16:0 was bound to CM and SM of DRMs but not of ROS, and the n-6 metabolite 22:4n-6 was greatly enriched in CM and SM of ROS. In CM of ROS, 18:0 was not detected, but it was present in ROS SM (38%). The only other lipid spot to show such segregation between ROS and DRMs was FFA, which contained no detectable 16:0 in ROS, but had 40% 16:0 in the DRMs. Moreover, as predicted by the fatty acid contents of total lipids (Fig. 4), there was more unsaturation of the ROS FFA pool, with only trace amounts of unsaturated fatty acids in the DRMs.

Comparison of DRMs and ROS Proteins

Compared to ROS parent membranes, DRMs are dramatically enriched in caveolin-1 (Fig. 1), a marker protein for specialized raft domains called caveolae. To compare the relative distribution of other photoreceptor proteins in ROS and DRMs, sucrose density gradients of ROS and DRMs were fractionated and equivalent volumes were subjected to SDS-PAGE and immunoblot analysis (Fig. 6). As we and others have reported, most of the detectable caveolin-1 was localized to low buoyant-density DRM fractions, along with a significant pool of transducin-α. In addition, a significant fraction of immunoreactivity against acylated, Src-family non–receptor tyrosine kinases, localizes to ROS-derived DRMs, as has been observed in other cell types, including brain. The full-length 48-kDa visual arrestin does not appear to fractionate to DRMs. However, the arrestin antibody used in our studies does not recognize the 44-kDa splice variant of arrestin, which has been shown to localize to DRMs isolated from light-adapted bovine ROS.

DISCUSSION

The most commonly used method for biochemical isolation of lipid rafts relies on the resistance of these domains to solubilization by nonionic detergents. Several years ago, Seno et al. isolated DRMs from bovine ROS and showed that these domains contain several proteins (e.g., transducin subunits and the cGMP-phosphodiesterase) involved in phototransduction. Many groups have continued to report on additional proteins that localize, at least partially, to ROS-derived DRMs. With the exception that DRMs from ROS are enriched in cholesterol and SM, surprisingly little is known about the lipid composition of these membrane domains. Given that the lipid environment is likely to be a major determinant of protein affinity and therefore of protein localization to DRMs, we undertook a detailed molecular analysis of the lipid species that populate ROS-derived membrane microdomains.

![Figure 3](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932933/)

**Figure 3.** Lipid composition of DRMs versus ROS. DRMs were quantitatively different from the parent ROS membrane. PC was resolved into three spots and DGs were resolved into two spots. PE, PS, PC2, and PC3 were more abundant in ROS, whereas PC1 and FFA were more abundant in DRMs. Data are expressed as are mole percents of all identified lipids in the purified extracts (mean ± SD, n = 5). Multivariate analysis of variance with post hoc Newman-Keuls test determined statistically significant differences between ROS and DRMs (P < 0.05). Levels of CM and SM were higher in DRMs but not significantly different from ROS (+).
Although there is strong evidence and independent confirmation that DRMs can be isolated from ROS membranes, the question arises as to how these biochemically derived domains relate to potential lipid rafts in living photoreceptors. In classic freeze-fracture/electron microscopy experiments, Andrews and Cohen observed PFPs in both plasma membranes and disks of ROS from mouse and frog. These patches were most abundant at the base of the outer segment and both filipin binding and saponin treatment suggested that PFPs were enriched in cholesterol. They described the PFPs observed in freeze-fracture replicas as “membrane regions of locally reduced fluidity that preferentially concentrate SM and cholesterol,” a definition that, for all practical purposes, describes lipid rafts and/or DRMs.

Several intriguing properties are shared between ROS-derived DRMs and PFPs observed in photoreceptors and in other cell types. First, ROS-derived DRMs are enriched in cholesterol and SM (Fig. 3) and PFPs observed in photoreceptors are enriched in cholesterol and, presumably, SM. Second, PFPs, by definition, are relatively devoid of proteins and the DRMs isolated in this study exhibited a more than threefold decrease in the protein-lipid ratio compared with the ROS membrane-starting material. Third, ROS-derived DRMs are dramatically enriched in a PC species (PC1, Figs. 2, 3) that is almost entirely saturated (Fig. 5), and the presence of PFP in bacterial membranes is associated with disaturated PC. Fourth, ROS-derived DRMs are dramatically enriched in caveolin (Fig. 6), as has been observed in PFPs from cultured cells. Although these results are consistent with the hypothesis that DRMs may represent biochemical isolates of PFPs, caution is merited in correlating these biochemical preparations with the in vivo composition and existence of lipid rafts. Nevertheless, our detailed analyses of the lipid composition of ROS-derived DRM may be useful in understanding the mechanisms of localization of a growing number of photoreceptor proteins to similarly prepared DRM fractions.

The composition of DRMs is dependent on the detergents chosen for their preparation, with Triton X-100 (used in our studies) suggested to be the most reliable for analyzing the association of molecules to DRMs. However, solubilization...
isozymes that, like other proteins, are localized to the DRMs.

Intriguingly, it was noted that nascent FFAs are poorly re-esterified. It will be interesting to determine whether there are phospholipase A actions govern their formation, but the cholesterol-rich PFPs result of lipid–lipid interactions or whether protein–lipid interactions could also play a role in the distribution of cholesterol. The role of cholesterol-binding proteins in regulating DRM cholesterol content in ROS-derived DRMs remains an open question.

Our experiments pose some fundamental questions about whether DRMs from ROS originate predominantly from disc or plasma membranes. If DRMs are biochemical isolates of PFPs, then it may be that DRMs originate from both ROS plasma membranes and basal disc membranes. This hypothesis is consistent with basal to apical cholesterol gradient observed in disks. With the exception of the newly synthesized basal disks, the cholesterol content of disc membranes is very low, thus reducing their “DRM-forming” potential. Although DRMs have been isolated from purified disks, it is unclear whether these DRMs originated primarily from basal disks. Based on our lipid composition data, the origin of DRMs (disc and/or plasma membrane) cannot be determined. However, our DRMs were prepared similarly to most reported photoreceptor DRMs preparations, thus providing potentially important information regarding the lipid environment conducive to DRM protein localization.

Raft Proteins

It has not been determined whether DRMs are formed as a result of lipid–lipid interactions or whether protein–lipid interactions govern their formation, but the cholesterol-rich PFPs observed by Andrews and Cohen were relatively devoid of protein. This is consistent with the relatively low abundance of the major ROS protein opsin in DRM fractions (Fig. 6, stained gel). Using reconstituted model membranes, Polozova and Lit-
man have suggested that rhodopsin’s association with dido-
cosahexaenoyl-PC and cholesterol’s affinity for dipalmitoyl-PC were essential factors in regulating lateral phase separation of lipids (i.e., raft formation). The presence of rhodopsin in a highly unsaturated lipid environment is consistent with its relatively decreased association with DRMs. However, it is conceivable that the palmitoylated cytoplasmic tail of rhodopsin could be inserted into the highly saturated DRMs, whereas the transmembrane domains of rhodopsin may lie in the relatively unsaturated, detergent-soluble membrane. Solubilization of cell membranes with Triton X-100 has been shown to extract “weakly associated” raft proteins.13 In this regard, if rhodopsin is peripherally associated with the DRMs via its palmitoylated C terminus, it may be extracted by the Triton X-100 concentrations used in our experiments.

The highly saturated lipid environment of DRMs favors localization of proteins acylated with saturated fatty acids, and several acylated proteins including heterotrimeric G-protein α subunits, Src family tyrosine kinases, and caveolins are recruited to raft membranes (reviewed in Resh). Both transducin-α11–13 and recoverin,15 two proteins modified by a heterogeneous pool of saturated and unsaturated fatty acids,44 associate with DRM domains. Regarding transducin-α, the degree of this saturation influences the affinity of the α subunit for ROS membranes.45 Given the highly saturated environment of ROS DRMs, we can speculate that the pool of transducin-α that localizes to these membrane domains is likely to be modified with saturated acyl chains (either 14:0 or 12:0). Supporting this speculation, the relative percentage of transducin-α that fractionates to DRMs in light-adapted ROS is ~30% of the total.41 This correlates well with the percentage of transducin alpha modified with 14:0 (~21%) and 12:0 (~12%).45 Although N-terminal acylation with saturated fatty acids may help target transducin (and perhaps other proteins) to DRMs, the acyl chain alone is unlikely to anchor the protein to the membrane.46 Interactions with DRM-associated proteins (e.g., caveolin-1) may be necessary to keep transducin-α within the DRMs.11 Figure 6 also shows that a significant portion of the Src family of tyrosine kinases is found within the DRM fraction. Heterogeneous acyl modifications of Src family kinases can regulate their association with raft domains,47 but at present, we do not know whether the Src family kinase(s) in ROS are heterogeneous acylated.

Although several proteins related to visual transduction localize, at least in part, to ROS-derived DRMs, the question arises as to whether these domains might be expected to promote phototransduction. Lipid rafts have been postulated to enhance, inhibit, and/or modulate a variety of signaling pathways (reviewed in Pike48). Based on the enrichment of cholesterol14 and highly saturated lipid, and the relative decrease in DHA, we speculate that the environment within DRMs does not promote active phototransduction. Rhodopsin activation is relatively inefficient in ROS membranes when cholesterol content is increased.59 Dietary depletion of DHA results in decreased rhodopsin activation, transducin-rhodopsin coupling efficiency, and PDE activation.50 Furthermore, Young and Albert51 have shown that apically displaced, “older” ROS disks, which are relatively depleted in cholesterol, bind transducin more efficiently than cholesterol-rich basal disks. They speculate that this is due to decreased rhodopsin activation, which is due to the increased cholesterol content of basal disks. Finally, transducin activation measured directly in DRMs is reduced when compared with ROS membranes.53 Taken together, these results suggest that membranes that are rich in cholesterol and saturated fatty acids and relatively depleted in DHA are less efficient at supporting active phototransduction. These speculations are based on the situation in which the signaling components reside within the lipid domain. It is conceivable that lipid rafts potentiate photoreceptor signaling by organizing the membrane components in such a way as to promote rapid protein–protein interactions outside the raft. This remains to be tested rigorously. In addition, it remains to be determined whether alternative signaling pathways (e.g., tyrosine phosphorylation- and/or phosphoinositide-based signaling) are potentiated within ROS-derived DRMs.

References


18. Pike LJ, Han X, Chung KN, Gross RW. Lipid rafts are enriched in arachidonic acid and plasmalogenethanolamine and their composition is independent of caveolin-1 expression: a quantitative elec-