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The Asymmetric Transmembrane Distribution of Phosphatidylethanolamine, Phosphatidylserine, and Fatty Acids of the Bovine Retinal Rod Outer Segment Disk Membrane

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Summary. The transmembrane distribution of the major aminophospholipids in the bovine retinal rod outer segment disk membrane, phosphatidylethanolamine and phosphatidylserine, was determined using a novel pair of permeable and impermeable covalent modification reagents. The values for the percentages of phosphatidylethanolamine and phosphatidylserine in the outer monolayer were calculated from a simple expression which takes into account the leakage of impermeable reagent into the disk lumen as monitored by the extent of labeling of lysine entrapped in the lumen. We infer from our results that at least 73 to 87% of the disk phosphatidylethanolamine and 77 to 88% of the disk phosphatidylserine are in the outer disk membrane monolayer. The fatty acid composition of the inner aminophospholipids is slightly more saturated than the outer aminophospholipids. Calculations using the lateral surface areas occupied by the disk membrane lipids suggest that 65 to 100% of the disk phosphatidylcholine is on the inner membrane surface. Since the disk phosphatidylcholine is also somewhat more saturated than the phosphatidylethanolamine and phosphatidylserine of the outer monolayer, the total inner membrane monolayer fatty acid composition is more saturated than that of the outer monolayer fatty acid composition.

The distribution of phospholipid classes between the inner and outer monolayers of several membrane bilayers has been determined by a number of diverse techniques [14, 17]. No general pattern or functional significance is as yet apparent [14, 17]. Reliable determinations of transmembrane phospholipid distribution may provide insight into its possible role in membrane function.

The transmembrane distribution of the aminophospholipids of the bovine retinal rod outer segment (ROS) disk membrane, phosphatidylethanolamine and phosphatidylserine, has been reported by four research groups. Using a permeable and impermeable pair of imidoesters as chemical probes, we preliminarily reported that at least 70% of the disk membrane aminophospholipids were exposed on the outer membrane surface [16]. Smith et al. [20] have reported that all aminophospholipids in the disk membrane are available for reaction with the impermeable reagent trinitrobenzenesulfonic acid. Recently, Crain et al. [3] using both trinitrobenzenesulfonic acid and the imidoester isethionylacetimidate (IAI), showed that at least 62–73% of the disk phosphatidylethanolamine and at least 25–31% of the phosphatidylserine are present in the disk membrane outer monolayer, while a minimum of 18–27% of the phosphatidylethanolamine and 25–35% of the phosphatidylserine are in the inner monolayer. Utilizing a very different approach, Sklar et al. [18, 19] found that the thermal behavior of disk membranes and lipid bilayers made from extracted ROS phospholipid classes could best be reconciled with a transmembrane phospholipid distribution which placed most of the aminophospholipids in one monolayer and most of the phosphatidylcholine and cholesterol in the other monolayer. Similar results are obtained and similar interpretations are made from differential scanning calorimetry studies of disk membranes and lipid bilayers [10, 11]. In contrast, Bonting and coworkers employing a combination of phospholipases and TNBS labeling find little or no asymmetry in the transbilayer distribution of phosphatidylethanolamine, phosphatidylserine, or phosphatidylcholine in the disk membrane [1, 6, 7].

We report here the transmembrane distribution

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of phosphatidylethanolamine and phosphatidylserine of the ROS disk membrane as derived by covalent labeling experiments using a pair of novel permeable and impermeable imidoesters. The advantages that these two reagents possess for chemical modification of biological structures are discussed at length elsewhere [13]. Using the labeling of lysine entrapped in the intradiskal space to measure the amount of "impermeable" labeling reagent gaining access to the inner membrane surface and compensating for this leakage with an analytical treatment similar to that of Nemes et al. [13], we find that at least 73 to 87% of the disk phosphatidylethanolamine and 77 to 88% of the phosphatidylserine are on the outer (cytoplasmic) disk membrane surface. We also present calculations which imply that phosphatidylcholine is predominantly located on the inner (noncytoplasmic) disk membrane surface. This transmembrane distribution of phospholipids is topologically identical to that found for the red blood cell, in which phosphatidylethanolamine is located on the inner (cytoplasmic) red blood cell membrane face and the choline-containing phospholipids are located on the outer (noncytoplasmic) face (for reviews *see* refs. 14 and 17). The fatty acid compositions of the inner and outer phosphatidylethanolamine are somewhat similar, as are the inner and outer phosphatidylserine fatty acid compositions, with the fatty acids of both inner phospholipids being slightly more saturated than those of the respective outer phospholipids.

Materials and Methods

Labeling Reagents

Isethionylacetimidate (IAI) was synthesized by a modification of the method of Whiteley and Berg [21]. (2-methylsulfonyl)ethyl acetimidate (SAI) and (2-acetimidoxy)ethyltrimethylammonium chloride (CAI) were synthesized as described in Nemes et al. [13].

Labeling Procedure

Retinal rod outer segments (ROS) were prepared essentially as described by Raubach et al. [15]. Disks were prepared by dialysis of ROS against hypotonic buffer, radioactive lysine was incorporated into the intradiskal space by prolonged incubation in a hypotonic medium, and the disks were resealed in isotonic salt, washed free of unincorporated lysine and labeled by either SAI, CAI or IAI, all as described previously [13]. Briefly, the labeling procedure consists of the addition of five portions of imidoester with each of these portions subdivided into a sequence of four smaller additions. Each of the five sequences of four additions is referred to as a reaction sequence. The membranes were washed free of reaction byproducts, hydrolysis products, and excess reagent after each reaction sequence. Aliquots of membrane were removed after each wash for the analysis of the extent of labeling of rhodopsin, lysine, phosphatidylethanolamine, and phosphatidylserine. The analysis of protein and lysine labeling are described in Nemes et al. [13].

Phospholipid and Fatty Acid Analyses

The procedure for the analysis of the extent of labeling of phosphatidylethanolamine and phosphatidylserine is summarized in Fig. 1. Aliquots withdrawn following the wash after each reaction sequence containing approximately 1 mg of rhodopsin were extracted by a modification of the method of Folch et al. [8, 12]. The extracts were transferred to a small volume of neat chloroform by rotary vacuum evaporation, applied as 1-cm bands to 20 × 20 cm silica gel plates and chromatographed in 65:25:5 chloroform/methanol/H_2O + 50 mg/liter butylated hydroxytoluene (BHT). The plate was then visualized under UV light after spraying with 0.4% dichlorofluorescein in methanol. Using this chromatographic solvent, the amidinated and unamidinated forms of phosphatidylethanolamine have essentially the same R_f; as do the amidinated and unamidinated forms of phosphatidylserine. The phosphatidylethanolamine and phosphatidylserine bands from each reaction sequence were each scraped from the plate into screw-capped test tubes. 1.4 ml of chloroform/methanol/water (65:50:5) plus 50 mg/liter BHT was added to each tube and the tubes were shaken. 100 µl of 0.5 M trinitrobenzenesulfonic acid in 5% $NaHCO_3$, pH 9, was added to each tube, the tubes were purged with argon, shaken vigorously, and incubated at 37° for 1 hr. Three ml of chloroform/methanol (2:1) + 50 mg/liter BHT and 1 ml of 0.1 mM EDTA were added to each tube and the tubes

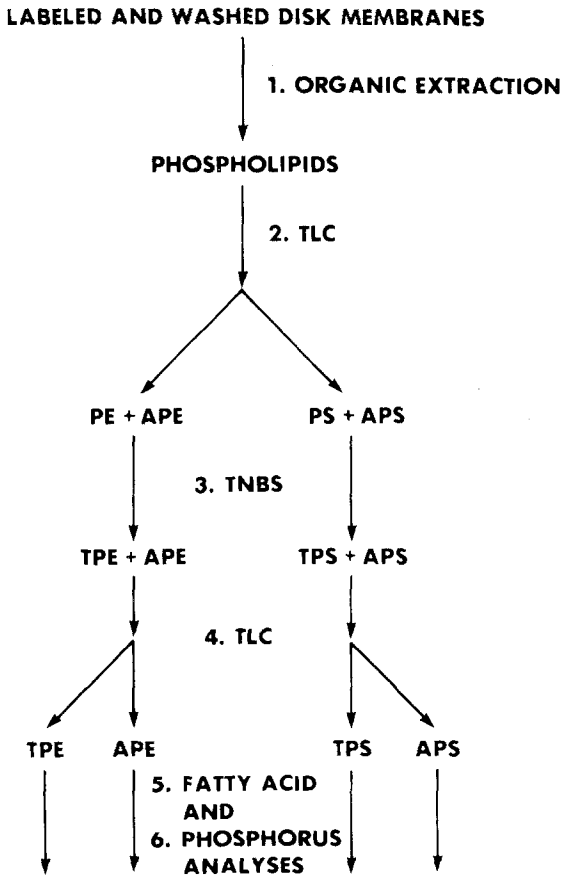

Fig. 1. Flow diagram of the analysis of the fatty acid and phosphorus content of amidinated and unamidinated (trinitrophenylated) phosphatidylethnolamine (APE and TPE) and phosphatidylserine (APS and TPS). *1* through *6* are the major manipulations in the procedure. The resulting product(s) of each manipulation are just below the corresponding arrowheads

were purged with argon, capped, shaken vigorously, and centrifuged for 5 min in a tabletop centrifuge at 1700 × g. The lower phases were collected, transferred to a small volume of chloroform by rotary vacuum evaporation, and further concentrated in a stream of argon. The concentrated samples were applied to 20 × 20 cm silica gel plates and rechromatographed in 65:50:5:3 chloroform/methanol/acetic acid/H_2O plus 50 mg/liter BHT. This solvent separates the amidinated phosphatidylethanolamine and amidinated phosphatidylserine from their respective trinitrophenylated analogs. The plates were visualized with dichlorofluorescein as above (in addition, the trinitrophenylated compounds were characteristically yellow) and the amidinated and trinitrophenylated phospholipids scraped into screw-capped tubes, each of which contained 14.7 μg heneicosanoic acid (21:0; twenty-one carbons, no double bonds) as an internal standard and 50 μg BHT in 100 μl chloroform. Fatty acid methyl esters were formed and analyzed by gas chromatography, and the phosphorus in each sample was determined as described previously [12].

Determination of Neutral Lipids

Lipids were extracted from ROS and transferred to a small volume of chloroform as above. The extract was chromatographed on 5 × 20 cm silica gel plates in hexane/diethyl ether/acetic acid, 30:60:1 plus 50 mg/liter BHT [4]. The chromatogram was visualized with dichlorofluorescein as above. With the aid of authentic standards, bands were identified as phospholipid, monoacylglycerides, diacylglycerides, free fatty acids, and triacylglycerides, and were scraped into screw-capped tubes containing BHT and heneicosanoic acid in chloroform. Methyl esters were formed, analyzed, and quantitated as described above and the relative concentrations of the components calculated.

Cholesterol was determined by a gas chromatographic procedure. The lower phase of a modified Folch extraction of ROS was dried over $MgSO_4$ and spun in a tabletop centrifuge to sediment the $MgSO_4$. The organic phase was removed, the $MgSO_4$ was washed with chloroform, and the combined organic phases were concentrated under a stream of argon. A known amount of 5β-cholestane-3β-ol was added as an internal standard, 100 μl of trimethylsilylimidazole in dry pyridine was added and the mixture was heated in a bead bath at 70° for 5 min. The derivatied cholesterol was analyzed on a Perkin-Elmer gas chromatograph equipped with a 5% Dexsil 400 column and programmed to increase the temperature from 190° to 270° at 8° per min. Known amounts of cholesterol were also derivatized and analyzed in a similar manner to determine the relative response factor for cholesterol and the internal standard.

Results and Discussion

Figure 2 shows the percent ROS disk phosphatidylethanolamine labeled by imidoester *vs.* reaction sequence. After the second sequence, the labeling of phosphatidylethanolamine by the permeable reagent SAI is virtually 100%. The labeling by the impermeable reagents CAI and IAI appear to saturate at approximately 92 and 85% of the total phosphatidylethanolamine, respectively. Figure 3 shows similar data for ROS phosphatidylserine. The SAI-labeled material is virtually 100% of the total after the second reaction sequence. The CAI-labeled phosphatidylserine plateaus at approximately 93% of total

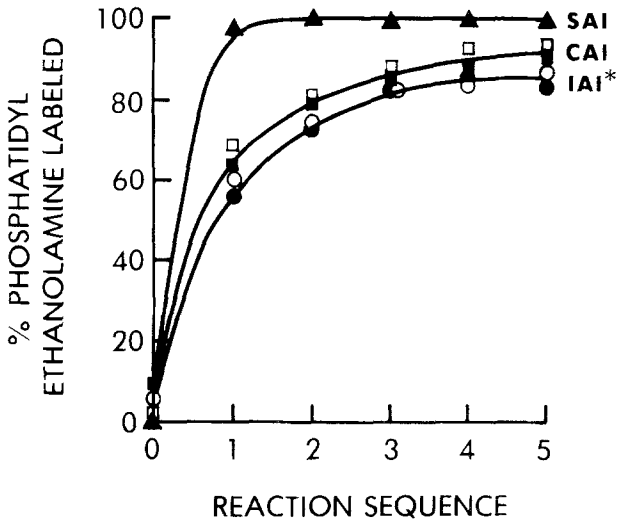

Fig. 2. The extent of amidination of ROS phosphatidylethanolamine by SAI (▲), CAI (■, □), or IAI (●, ○). Open symbols indicate extent of labeling assayed by fatty acid analysis, and solid symbols indicate extent of labeling assayed by phosphorus analysis of appropriate TLC bands. (*IAI labeling in this figure and in Fig. 3 were each determined from different experiments and are not directly comparable unless corrected for the different membrane permeabilities in each preparation.[1])

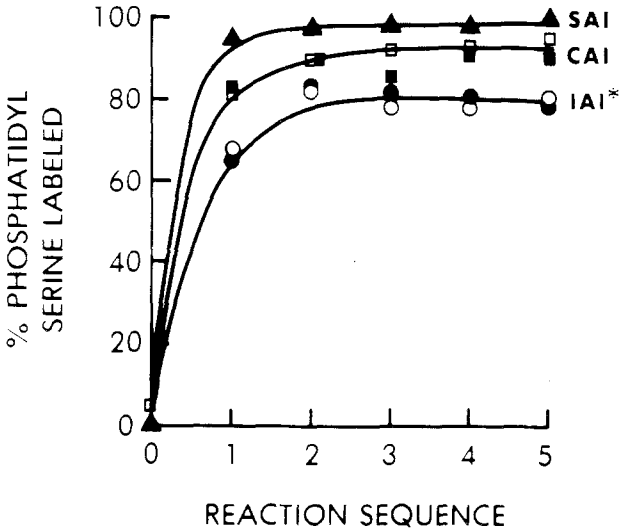

Fig. 3. The extent of amidination of ROS phosphatidylserine by SAI (▲), CAI (■, □), or IAI (●, ○). Open symbols indicate labeling assayed by fatty acid analysis, and solid symbols indicate labeling assayed by phosphorus analysis. (*The IAI labeling in this figure and in Fig. 2 were each determined from different experiments and are not directly comparable unless corrected for the different membrane permeabilities in each preparation.[1])

phosphatidylserine and the IAI-labeled material plateaus at approximately 80%. (The IAI labeling results for phosphatidylethanolamine and phosphatidylserine were taken from two separate experiments in which the membrane preparations had different permeabilities and therefore are not directly com-

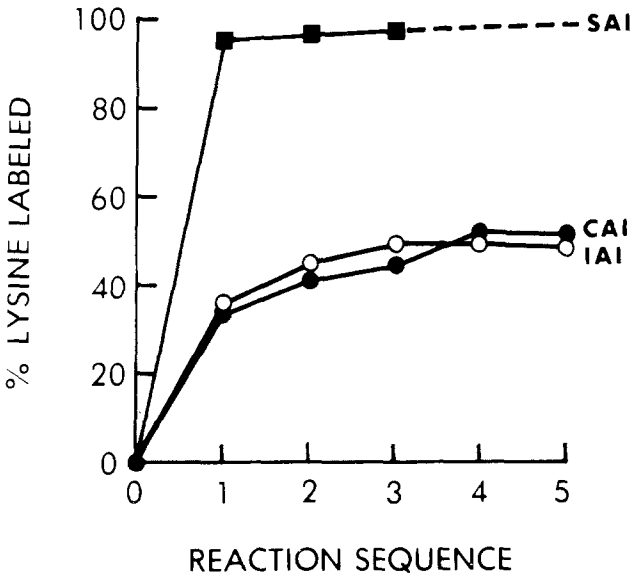

Fig. 4. Imidoester labeling of tritiated lysine entrapped in ROS disk membranes *vs.* reaction sequence. The details of the lysine labeling are given in Nemes et al. [13]

parable.[1]) Because the "impermeable" reagents are actually somewhat permeable, these data are not a direct measure of the percentage of phospholipid on the outer membrane surface. These data must be adjusted for the fraction of phospholipids of the interior surface which are labeled by reagent which has leaked into the disk. The details of the treatment of the data are discussed in Nemes et al. [13]. Note that this treatment assumes that exchange, or "flip-flop", of phosphatidylethanolamine or phosphatidylserine between the inner and outer monolayers is negligible on the time scale of our labeling experiments (ca. 4–5 hr). The presence or absence of lipid flip-flop in the disk membrane has yet to be determined. Briefly, labeling of the amino groups of lysine entrapped in the disk lumen is used to monitor the amount of leakage of imidoester. The percentage of internal lysine labeling *vs.* reaction sequence for the experiments presented in Figs. 2 and 3 is given in Fig. 4. As expected, the SAI labeling modifies nearly 100% of the internal lysine after the second reaction sequence. The saturation of lysine labeling at the third or fourth reaction sequence for both the CAI and IAI experiments indicates the presence of more than one population of lysine-containing disks. Of the disks containing lysine, 50% are essentially completely impermeable to labeling reagent, while the remaining 50% are eventually completely permeable to reagent.

In addition, the amount of lysine retained by the disks after extensive incubation in lysine-containing medium and washing prior to labeling indicates that there is a third population of disks that must also be taken into account for the treatment of the raw labeling data. The lysine contained in the total disk internal volume at the start of the labeling procedure is about 80% of the lysine content of the same volume of lysine-containing incubation medium. Thus approximately 20% of the disks are either (1) rapidly leaky to lysine and thus very likely to be totally permeable to the "impermeable" imidoesters and actually do represent a third class of disks, or (2) are totally impermeable to lysine and thus are likely to be also impermeable to the impermeable imidoesters. An expression taking into account these classes of disks in interpreting the labeling data has been formulated as follows:

$$PL_L^i = x_O^i PL_O + (x_I^i D_{RL} + x_I^i D_{SL}) PL_I$$

where: PL_L = the fraction of aminophospholipid labeled; PL_O = the fraction of aminophospholipid actually on the outer membrane surface; PL_I = the fraction of aminophospholipid actually on the inner membrane surface; x_O = the fraction of outer amino groups actually labeled; x_I = the fraction of inner amino groups actually labeled on the subset of the disks which are permeable to reagent. At saturation (i.e., effectively the last or next to last reaction sequences) it is assumed that $x_O = 1$ and $x_I = 1$. D_{RL} = the fraction of the disks that are rapidly leaky to reagent; D_{SL} = the fraction of the disks that are slowly leaky to reagent on the timescale of our experiments and i = the number of reaction sequences. Note that $PL_O + PL_I = 1$. Assuming that $x_O^5 = 1$ and $x_I^5 = 1$ (i.e., when labeling has achieved saturation) and substituting and rearranging we obtain an expression for the fraction of aminophospholipid on the outer membrane surface:[2]

$$PL_O = \frac{PL_L - (D_{RL} + D_{SL})}{1 - (D_{RL} + D_{SL})}. \quad (1)$$

[1] The plateau level of labeling of entrapped lysine varies somewhat from preparation to preparation, implying a corresponding variability in the leakiness of different preparations to labeling reagent. For this reason, raw labeling data from different experiments must be corrected for reagent leakage before a direct comparison from one experiment to another can be made (*see* Results and Discussion).

[2] This treatment is similar but not identical to case *c*), Eqs. (5) and (6) in the appendix of ref. 13, where rhodopsin labeling is considered. The labeling of phospholipid accessible to reagent appears to saturate more slowly than that of the rhodopsin amino groups. Thus, the assumption that the external amino groups are essentially saturated at all reaction sequences ($X_O^i = 1$) is appropriate for rhodopsin labeling (as used in ref. 13) but is not satisfactory for the treatment of the phospholipid labeling in the present paper. The present analysis differs from that in ref. 13 in assuming saturation of the outer phospholipid only at the final reaction sequence ($X_O^5 = 1$) and in assuming that the inner phospholipids of

Note that for a given D_{SL}, the larger the D_{RL}, the smaller the estimate of PL_O. Thus, the maximum reasonable estimate of D_{RL} will yield a minimum limit to PL_O. As stated above, D_{RL} could be as much as 0.2 prior to labeling. During the course of the labeling process, 24% of the lysine entrapped in the disks is released into the medium [13]. Whether this released lysine is amidinated or unamidinated will affect the calculated value of PL_O. For the purposes of establishing an upper limit for D_{RL} and a minimum value for PL_O, we assume that the loss of lysine is from rapidly leaky disks created during the labeling procedure. Therefore, an additional 20% of the total disk population ($0.24 \times 80\%$ lysine-containing disks) may become totally leaky as a result of experimental manipulation, and the maximum value for D_{RL} is 0.4. It follows that the minimum value for $D_{NL}+D_{SL}$ is 0.6 (where D_{NL} is the fraction of nonleaky disks). Since the lysine labeling saturates at 50%, the amount of nonleaky disks, D_{NL}, is equal to the amount of slowly leaky disks, D_{SL}, assuming that the lysine labeling reflects only these vesicle types. Thus, for a maximum D_{RL} of 0.4, the value of D_{SL} is 0.3. Using these values and a value of $PL_L=0.92$ for phosphatidylethanolamine, Eq. (1) yields a *minimum* PL_O of 0.73. Therefore, a *minimum* of 73% of the total phosphatidylethanolamine is on the outer disk membrane surface. Using 0.93 for the PL_L for phosphatidylserine, Eq. (1) yields a *minimum* PL_O of 0.77 or a *minimum* of 77% of the disk phosphatidylserine being on the outer disk surface. These two values are derived from CAI-labeling results. Since CAI and IAI show virtually identical leakages across the disk membrane, this implies that CAI labels the ROS disk membrane aminolipids somewhat more effectively than IAI. This is not surprising in light of the fact while CAI is positively charged, IAI, like the disk membrane, bears a negative charge. Since both the CAI and IAI results each in themselves provide a minimum value for the fractions of aminophospholipids on the outer membrane surface, the higher of these, those derived from the CAI-labeling results, are more accurate estimates of the true values of PL_O for phosphatidylserine and phosphatidylethanolamine.

If minimum values for D_{RL} and for D_{SL} are used, Eq. (1) will yield a maximum estimate for PL_O. As mentioned above, the 20% of disks which fail to incorporate lysine could be impermeable to both lysine and imidoester. In addition, it is conceivable that the 24% of lysine released during the labeling procedure might not reflect disks leaky to imidoester. In this case D_{RL} would be zero and D_{SL} would be 0.4. Substitution of these values into Eq. (1) along with $PL_L=0.92$ for phosphatidylethanolamine yields a maximum estimate of PL_O of 0.87 for phosphatidylethanolamine. A similar calculation employing $PL_L=0.93$ for phosphatidylserine gives $PL_O=0.88$. Thus the amount of phosphatidylethanolamine in the outer monolayer of the disk membrane is approximately 73 to 87% and the amount of phosphatidylserine in the outer monolayer is about 77 to 88%. The value for phosphatidylethanolamine on the outer surface is in good agreement with that of Crain et al. [3]. However, our value for phosphatidylserine is substantially higher than theirs. Apparently they were not able to modify a large fraction of external phosphatidylserine under their labeling conditions. Recently, Drenthe and coworkers have used phospholipases A, C and D [6] and phospholipase D in combination with trinitrobenzenesulfonic acid [7] to assess the transmembrane distribution of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine in the disk membrane. These authors find only a slight asymmetry for phosphatidylcholine (40–45% in the outer monolayer) and phosphatidylethanolamine (55–60% in the outer monolayer) and no asymmetry for phosphatidylserine. The reason for the discrepancy between our results and theirs is not apparent at this time.

The fatty acid compositions of the amidinated and unamidinated phosphatidylethanolamine and phosphatidylserine were determined for the CAI-labeling experiment. The phosphatidylethanolamine and phosphatidylserine that were not labeled consist entirely of phosphatidylethanolamine and phosphatidylserine on the inner surface of the membrane. However, the labeled phosphatidylethanolamine and phosphatidylserine consists of both phosphatidylethanolamine and serine on the inner surface of leaky disks and phosphatidylethanolamine and serine on the outer surface of all disks. Since the fatty acid compositions of phosphatidylethanolamine and phosphatidylserine are known directly from analyses of the unamidinated controls and the relative amounts of inner and outer aminophospholipid in the labeled aminophospholipid fractions are easily calculated, the fatty acid compositions of the outer surface aminophospholipids can be determined. Table 1 gives the fatty acid composition of inner and outer

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the leaky vesicles ($D_{RL}+D_{SL}$) are also saturated at the final reaction sequence ($X_I^5=1$). To emphasize the difference in the analyses of the protein and the lipid labeling, a somewhat different notation is used. Each of the symbols in the present treatment can be identified with a corresponding symbol in ref. 13, except one that differs in definition. In ref. 13, X_I^i refers to the fraction of inner amino groups which are labeled on the subset of vesicles which are leaky to reagent. Since the lysine labeling plateaus (Fig. 4), leaky vesicles here refers to those which are rapidly leaky (D_{RL}) as well as those which are slowly leaky (D_{SL}) on the timescale of our experiments

Table 1.

Fatty acid	Phosphatidylethanolamine	
	% of inner	% of outer
16:0	14.8	11.0
18:0	23.3	25.1
18:1	6.0	3.8
20:4	3.5	3.6
22:5[a]	3.0	3.8
22:6	36.5	48.1
Other	12.9	4.6
	Phosphatidylserine	
16:0	7.2	0.6
18:0	21.0	21.7
18:1	5.8	1.6
20:4	3.4	1.1
22:4	4.1	3.5
22:5[a]	3.6	6.0
22.6	34.2	48.4
24:4	4.1	5.6
24:5	4.2	9.1
Other	12.4	2.4

[a] 22:5 represents the sum of 22:5ω3 and 22:5ω6 species.

Table 2.

Lipid class	Moles/mole rhodopsin
Phosphatidylethanolamine	31.6±1.3
Phosphatidylserine	10.7±0.7
Phosphatidylcholine	29.6±0.9
Phosphatidylinositol	1.4±1.0
Sphingomyelin	1.0±0.2
Other phospholipid	0.8±0.6
Cholesterol	8.2±1.1
Free fatty acid	6.1
Diacylglyceride	0.8
Monoacylglyceride	0.3

phosphatidylethanolamine and phosphatidylserine; the outer compositions were calculated by using a value of 80% for the percentage of both phosphatidylethanolamine and phosphatidylserine on the outer membrane surface. The fatty acid compositions are similar except that for both phospholipids, the inner composition is relatively enriched in more saturated fatty acids, particularly 16:0, and relatively depleted in polyunsaturated acids, particularly 22:6. Since the other major ROS disk membrane phospholipid, phosphatidylcholine, is likely to be predominantly present in the inner membrane monolayer [11, 18, 19] (*see* below) and is also somewhat more saturated than the ROS outer phosphatidylethanolamine and phosphatidylserine [15], the inner monolayer possesses a more saturated complement of fatty acids than the outer monolayer.

Table 2 shows the total lipid composition of the ROS disk membrane. Using these values and two simple assumptions, it is possible to estimate the transmembrane distribution of phosphatidylcholine in the disk membrane. The first assumption is that the total lateral area occupied by lipid in the outer membrane monolayer is approximately equal to the total lateral area occupied by lipid in the inner membrane monolayer [5]. Since rhodopsin comprises at least 95% of the integral protein of the disk membrane [9] and since numerous studies suggest that rhodopsin is an approximately cylindrical molecule which spans the disk membrane, this seems a reasonable assumption. The second assumption is that the lateral areas occupied by each ROS phospholipid and diacylglyceride molecule in the membrane are approximately equal and that the lateral areas of each free fatty acid, monoacylglyceride, and cholesterol are approximately equal and equal to half that of a phospholipid. The maximum percentage of phosphatidylcholine on the outer surface of the disk membrane may then be calculated if, in the extreme, all ROS diacylglyceride, monoacylglyceride, minor phospholipids, free fatty acid, cholesterol, and the maximum calculated amounts of inner phosphatidylethanolamine and phosphatidylserine are all assumed to be present in the inner disk membrane monolayer, while the minimum calculated amounts of outer phosphatidylethanolamine and phosphatidylserine are assumed to be in the outer monolayer. Using these parameters, we calculate that maximally 35% of the phosphatidylcholine is in the outer monolayer. If either all minor disk membrane lipid components were assumed to be in the outer monolayer or if the maximum values of outer phosphatidylethanolamine and phosphatidylserine were used, virtually all ROS phosphatidylcholine would necessarily reside on the inner membrane surface.

In summary, we infer from these chemical labeling studies that 73 to 87% of the phosphatidylethanolamine and 77 to 88% of the phosphatidylserine are in the outer membrane monolayer, while 65 to 100% of the phosphatidylcholine exists in the inner membrane monolayer. The inner membrane monolayer appears to possess a slightly more saturated fatty acid composition than the outer monolayer. Note also that studies of the thermal behavior of ROS disk membranes of bilayers made from classes of extracted ROS disk phospholipid, using both parinaric acid fluorescence polarization [18, 19] and differential scanning calorimetry [11, 12], suggest that over half of the ROS cholesterol is present in the inner membrane monolayer, if it is assumed that rhodopsin has little effect on the thermal properties of the disk membrane lipids. The latter assumption

Table 3.

Lipid	Outer monolayer	Inner monolayer	Total lipids/ rhodopsin in both monolayers
Phosphatidylethanolamine	77–87%	13–27%	32
Phosphatidylserine	77–88%	12–23%	11
Phosphatidylcholine	0–35%	65–100%	30
Cholesterol	≦50%	≧50%	8
Total fatty acids	More unsaturated	More saturated	

is supported by recent proton [2], carbon [2], phosphorus (A.T. Deese, M.F. Brown, and E.A. Dratz, *submitted*) and deuterium (A.J. Deese, E.A. Dratz, F.W. Dahlquist, and M.R. Paddy, *submitted*) NMR studies of rhodopsin-lipid interactions. The approximate transmembrane distributions of the major lipids of the ROS disk membrane are summarized in Table 3.

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