Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 12130–12134, December 1994
Biochemistry

Interactions between saturated acyl chains confer detergent resistance on lipids and glycosylphosphatidylinositol (GPI)-anchored proteins: GPI-anchored proteins in liposomes and cells show similar behavior

(membrane domain/model membrane/liquid-ordered phase/glycosphingolipid/cholesterol)

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Communicated by William J. Lennarz, August 22, 1994 (received for review June 14, 1994)

ABSTRACT **Proteins anchored by GPI are poorly solubilized from cell membranes by cold nonionic detergents because they associate with detergent-resistant membranes rich in cholesterol and sphingolipids. In this study, we demonstrated that cholesterol and sphingolipid-rich liposomes were incompletely solubilized by Triton X-100. GPI-anchored placental alkaline phosphatase incorporated in these liposomes was also not solubilized by cold Triton X-100. As sphingolipids have much higher melting temperatures (T_m) than cellular phospholipids, a property correlated with T_m might cause detergent inextractability. In support of this idea, we found that the low-T_m lipid dioleoyl phosphatidylcholine (DOPC) was efficiently extracted from detergent-resistant liposomes by Triton X-100, whereas the high-T_m lipid dipalmitoyl phosphatidylcholine (DPPC) was not. The fluorescence polarization of liposome-incorporated diphenylhexatriene was measured to determine the "fluidity" of the detergent-resistant liposomes. We found that these liposomes were about as fluid as DPPC/cholesterol liposomes, which were present in the liquid-ordered phase, and much less fluid than DOPC or DOPC/cholesterol liposomes. These findings may explain the behavior of GPI-anchored proteins, which often have saturated fatty acyl chains and should prefer a less-fluid membrane. Therefore, we propose that acyl chain interactions can influence the association of GPI-anchored proteins with detergent-resistant membrane lipids. The affinity of GPI-anchored proteins for a sphingolipid-rich membrane phase that is not in the liquid crystalline state may be important in determining their cellular localization.**

Membranes that contain GPI-anchored proteins have been isolated from cultured epithelial cells (1, 2), fibroblasts (2), and lymphocytes (3) on the basis of their surprising insolubility in nonionic detergents such as Triton X-100. These membranes are rich in sphingolipids and cholesterol (1) and contain several proteins in addition to those anchored by GPI (2, 4, 5). These include caveolin, a marker for 50-nm-diameter non-clathrin-coated plasma membrane invaginations called caveolae (6). This and other studies have led to the suggestion that caveolae can be isolated from cells by detergent extraction (7, 8).

It is not known how the lipids and proteins in these membranes allow them to remain associated with each other in the presence of detergent. Bilayers consisting of phospholipids and glycosphingolipids contain compositionally distinct domains (reviewed in ref. 9). It has been suggested that the same interactions that cause glycolipids to form clusters may cause their detergent resistance (1, 10). There is evidence to suggest that hydrogen bonds between glycosphingolipids may help form membrane domains (11). Alternatively, or in addition, acyl chain interactions may be important in this process, as glycolipid-rich domains can be present in a gel-like state (9). We have tested these models by determining the detergent solubility of artificial liposomes, with or without GPI-anchored proteins, and by investigating the physical properties of detergent-resistant liposomes.

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METHODS

Liposome Preparation and Detergent Extraction. Purity of all lipids was checked by TLC. Lipids (2 mg) in solvent were dried under N_2 and then dissolved in 1 ml of 60 mM octyl glucoside in phosphate-buffered saline (PBS) (1) by sonication. Liposomes formed by dialysis against PBS were placed on 5–20% sucrose gradients in PBS and subjected to ultracentrifugation at 200,000 × *g* for at least 12 hr at 4°C. Thirty-five microliters of 10% (vol/vol) Triton X-100 in PBS, or PBS alone, was added to half the liposomes and incubated on ice for 20 min. After centrifugation at 200,000 × *g* for 2 hr at 4°C, lipids isolated from the pellets were separated by high-performance thin-layer chromatography (HPTLC) and quantitated by charring (12). Protein-free sphingolipid/cholesterol-rich liposomes (SCRL) were 1:1:1:1:2 (mol ratio) bovine liver phosphatidylcholine (bPC)/bovine liver phosphatidylethanolamine (bPE)/brain sphingomyelin (SM)/brain cerebrosides (CB)/cholesterol (Chol) (Avanti Polar Lipids and Sigma). SCRL formed a single band on sucrose density gradients. SCRL containing brominated dioleoyl PC instead of bPC also formed one band on these gradients, and this band was shifted to the appropriate density. Control experiments showed that >90% of the lipid that could be solubilized in 24 hr was solubilized in 20 min. In addition, >95% of the lipid that could be pelleted in 2.5 hr was pelleted in the first 30 min of centrifugation. For convenience, the shorter times of centrifugation and solubilization were used in later experiments.

Purification of Placental Alkaline Phosphatase (PLAP). One milligram of PLAP (Sigma) was dissolved in 1% (vol/vol) Triton X-114 in PBS and subjected to phase separation. Two hundred microliters of 35% (wt/vol) 3-[(3-cholamidopropy-

Abbreviations: SCRL, sphingolipid/cholesterol-rich liposomes; DOPC, dioleoyl phosphatidylcholine; DPPC, dipalmitoyl phosphatidylcholine; GPI, glycosylphosphatidylinositol; bPC, bovine phosphatidylcholine; bPE, bovine phosphatidylethanolamine; SM, sphingomyelin; CB, cerebrosides; Chol, cholesterol; DPH, diphenyl-1,3,5-hexatriene; PLAP, placental alkaline phosphatase; TCA, trichloroacetic acid; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; PI-PLC, phosphatidylinositol-specific phospholipase C; HPTLC, high-performance thin-layer chromatography; l_o, liquid ordered.

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l)dimethylammonio]-1-propanesulfonate (CHAPS) in PBS was added to the detergent phase and incubated overnight at 4°C. The protein was loaded on a Sephacryl S200 column preequilibrated with PBS/2% CHAPS. PLAP enzymatic activity was detected in selected fractions by using a kit (Sigma). Fractions containing PLAP, but not Triton X-114, from nine similar preparations were pooled and stored at 4°C. The specific activity of the purified PLAP was 1123 units/mg, a 70-fold purification over the starting material. Approximately 1% of the protein in the starting material was recovered as GPI-anchored PLAP, similar to the value obtained by Udenfriend and coworkers (13). In experiments to characterize PLAP, recombinant *Bacillus cereus* phosphatidylinositol-specific phospholipase C (PI-PLC) was isolated from overexpressing *Escherichia coli* (provided by J. Volwerk) (14). Immunoprecipitation using rabbit anti-human PLAP antibodies (Dako) was as described (5).

Preparation of Liposomes Containing PLAP. Lipids in SCRL with PLAP were 2:1:1:2 (mol ratio) bPC/SM/CB/Chol. After dialysis, liposomes containing 2 mg of lipids were subjected to three freeze–thaw cycles. Liposomes and 200 ng of purified PLAP were mixed, dialyzed against PBS, and subjected to sucrose gradient centrifugation. Liposomes were dialyzed against PBS, collected by centrifugation at 200,000 × *g* for 1 hr, and washed three times in PBS. About half of the PLAP associated stably with liposomes. Liposomes were resuspended in 350 μl of PBS or 1% Triton X-100 in PBS, incubated on ice, and collected by centrifugation. PLAP in supernatant and pellet fractions was precipitated with 10% (wt/vol) trichloroacetic acid (TCA) and detected by Western blotting and enhanced chemiluminescence (ECL; Amersham) (5).

Diphenyl-1,3,5-hexatriene (DPH) Polarization. To measure polarization, 1 μl of 24 μM DPH dissolved in tetrahydrofuran was added to 1 ml of liposomes (1 mg/ml) in PBS. After incubation for 1 hr at room temperature in the dark, DPH fluorescence was measured on a Spex 212 spectrofluorimeter (Spex Industries, Edison, NJ) equipped with Glan–Thompson polarization filters. Fluorescence intensity was measured for periods of 1 sec. Samples were incubated in the dark for 1 min between successive fluorescence readings (in which the filters were set in various vertical and horizontal orientations) to minimize DPH photoisomerization effects. The fluorescence of background samples lacking DPH was found to be negligible. Polarization was calculated with the equation

$$P = I_{VV} - (I_{HV}/I_{HH})I_{VH}/[I_{VV} + (I_{HV}/I_{HH})I_{VH}],$$

where I is emission intensity and the subscripts refer to the orientation (V, vertical; H, horizontal) of polarization filters in the excitation and emission beams, respectively. Polarization was measured successively at 37°C, 24°C, and 15°C by incubating samples in a water bath at the desired temperature until shortly before the fluorescence was read in a sample chamber adjusted to the same temperature.

RESULTS

Sphingolipid-Enriched, Protein-Free Liposomes Are Not Fully Solubilized by Triton X-100. Membranes isolated by detergent extraction of Madin–Darby canine kidney (MDCK) cultured epithelial cells contain glycerophospholipids, sphingolipids, and Chol in a mole ratio of approximately 1:1:1 (1). To determine whether proteins were required for the detergent insolubility of these membranes, we subjected artificial liposomes with a similar lipid composition, called SCRL (see *Methods*), to detergent extraction. Control liposomes were prepared that contained 7:1 bPC/Chol.

All the experiments described here used the inability of lipids to pellet during ultracentrifugation as a criterion of solubilization. Although it is likely that any sedimentable lipids are not solubilized, the inability to sediment does not guarantee solubilization. For convenience, however, we will refer to pelletable material as "insoluble," and material that remains in the supernatant after ultracentrifugation as "soluble." We will also refer to the conversion by Triton X-100 of lipids to a form in which they no longer sediment as "detergent extraction."

Pelleted lipids were analyzed by HPTLC (Fig. 1). Both bPC and Chol in control liposomes were largely solubilized by Triton X-100 (Fig. 1*A*). The lipids in SCRL were solubilized differentially. Only 25% of the phospholipids resisted solubilization, while 82% of the CB, 86% of the sphingomyelin, and 63% of the Chol could be pelleted after extraction (Fig. 1*B* and Table 1). Thus, bPC and bPE are more easily solubilized from SCRL than any of the other lipids. This result shows that interactions between the lipids are sufficient for detergent insolubility.

Triton insolubility might require a particular mixture of CB, SM, and Chol. Alternatively, one or more of these lipids might be especially important. To address this question, we made a series of liposomes with simpler lipid compositions. The detergent solubility of each is shown in Table 1.

To determine whether CB and SM played equal roles in the insolubility of SCRL, we constructed liposomes containing either 1:1:1 PC/CB/Chol or 1:1:1 PC/SM/Chol. Triton solubilized the lipids from both of the liposomes to the same extent as from SCRL, showing that CB and SM behave identically under these conditions. The remaining liposomes examined contained 80–90% bPC and one or two additional lipids. Sixty percent of the CB in 9:1 bPC/CB liposomes was insoluble in Triton X-100. By contrast, only 11% of the SM was insoluble in 9:1 bPC/SM liposomes, and only 1% of the Chol in 9:1 bPC/Chol liposomes was resistant to extraction. This result shows that CB does not require Chol for insolubility and that CB has a stronger propensity than SM to resist extraction when present at a low concentration in the membrane. However, a higher proportion, 28%, of the SM remained insoluble when 18:1:1 PC/CB/SM liposomes were extracted. Therefore, SM may partially resist extraction by associating with CB. The inclusion of 10% Chol in either bPC:CB or bPC:SM liposomes, to form liposomes that were

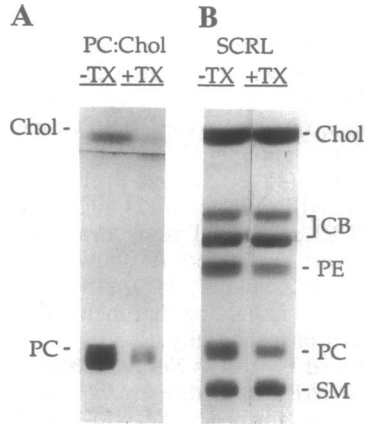

FIG. 1. Liposome solubility in Triton X-100. Liposomes [7:1 PC/Chol (*A*) or SCRL (*B*)] were extracted on ice with Triton X-100 (+TX) or incubated without detergent (−TX) and subjected to ultracentrifugation. Lipids in the pellets were analyzed by HPTLC and densitometry after charring. In *A*, three times as much of the pelleted lipids from the +TX sample as from the −TX sample was analyzed. *B* shows equal fractions of the pelleted lipids from both samples.

Table 1. Triton X-100 insolubility of liposome lipids

Initial liposome lipid composition	Sedimentability of each lipid			
	PC	CB	SM	Chol
PC/PE/CB/SM/Chol, 1:1:1:1:2 (SCRL) (7)	24 ± 9 26 ± 19*	82 ± 20	86 ± 9	63 ± 11
PC/CB/Chol, 1:1:1 (3)	40 ± 3	78 ± 19	–	61 ± 8
PC/SM/Chol, 1:1:1 (3)	29 ± 4	–	69 ± 3	84 ± 3
PC/CB, 9:1 (6)	12 ± 8	60 ± 19	–	–
PC/CB/Chol, 8:1:1 (3)	3 ± 1	69 ± 11	–	18 ± 14
PC/SM, 9:1 (4)	11 ± 7	–	11 ± 6	–
PC/SM/Chol, 8:1:1 (4)	4 ± 2	–	10 ± 3	14 ± 4
PC/CB/SM, 18:1:1 (5)	4 ± 1	60 ± 19	28 ± 7	–
PC/Chol, 9:1 (4)	2 ± 1	–	–	1 ± 2
DPPC/Chol, 2:1 (2)	96 ± 2†	–	–	100 ± 1
DOPC/Chol, 2:1 (2)	1 ± 1†	–	–	1 ± 1

Liposomes incubated with or without Triton X-100 on ice were subjected to ultracentrifugation. Pelleted lipids were recovered and quantitated by HPTLC, and the insoluble fraction of each lipid is shown [(% of each lipid pelleted after detergent extraction/% pelleted after mock extraction) × 100] (mean ± SD). The number of repetitions of each experiment is indicated in parentheses. Liposomes of the following compositions were alternatively prepared by resuspension of dried lipids in PBS, bath sonication for 1 hr, and three freeze–thaw cycles: SCRL; DPPC; DOPC; DPPC/Chol, 9:1; DOPC/Chol, 9:1; bPC/SM/CB, 18:1:1; bPC/Chol/SM, 8:1:1 (data not shown). Results were similar to those shown.

*Value for PE.

†As DPPC does not char on HPTLC plates, both of these values were determined with radioactive tracers (Fig. 2 and associated text).

8:1:1 bPC/sphingolipid/Chol, did not affect the solubility of either sphingolipid.

Melting Temperature Correlates with Lipid Insolubility. These results suggested that CB has the greatest tendency to resist extraction by detergent, SM has an intermediate tendency to do so, and brain PC is largely solubilized. This order correlates with the gel/crystal → liquid crystal melting temperatures of these lipids. Some pure CB may not form bilayers. However, analysis of bilayer-forming hydrated brain CB by x-ray diffraction (15) or differential scanning calorimetry (16) shows that these lipids undergo a melting transition at 55–65°C. Brain SM has a melting temperature range of 30–45°C (17). bPC melts below 0°C. Thus, it seemed that a property associated with high melting temperature might be important in the detergent insolubility of lipids. Alternatively, phospholipids may be inherently more soluble than sphingolipids.

To distinguish between these possibilities, we selected a phospholipid with monounsaturated fatty acid chains, dioleoyl PC (DOPC; melting temperature, −20°C), and one with saturated chains, dipalmitoyl PC (DPPC; melting temperature, 41°C). Trace amounts (1–2 × 10^4 cpm) of [*N*-methyl-[^{3}H]choline]DPPC (40–80 Ci/mmol; 1 Ci = 37 GBq) or [1,2-di[1-^{14}C]oleoyl]DOPC (100–124 mCi/mmol) were incorporated into SCRL, and the Triton extraction procedure and centrifugation were performed. As shown in Fig. 2*A*, 76% of the [^{3}H]DPPC (bar 2), but only 24% of the [^{14}C]DOPC (bar 4), remained insoluble, indicating that melting temperature can be a determinant of insolubility.

A GPI-Anchored Protein Is Not Solubilized from SCRL by Triton X-100. PLAP was purified (*Methods* and Fig. 3) and incorporated into preformed SCRL (18). The Triton solubility of PLAP in SCRL or in 7:1 PC/Chol liposomes was then analyzed. Most PLAP was solubilized from the control liposomes (Fig. 4 *Left*), but 85 ± 9% (n = 5) of PLAP in SCRL could be pelleted after treatment with Triton X-100 (Fig. 4 *Right*). This shows that GPI-anchored proteins associate directly with detergent-resistant lipids in detergent-resistant membranes. Fatty acyl chains of GPI-anchored proteins are more saturated than those found in membrane phospholipids

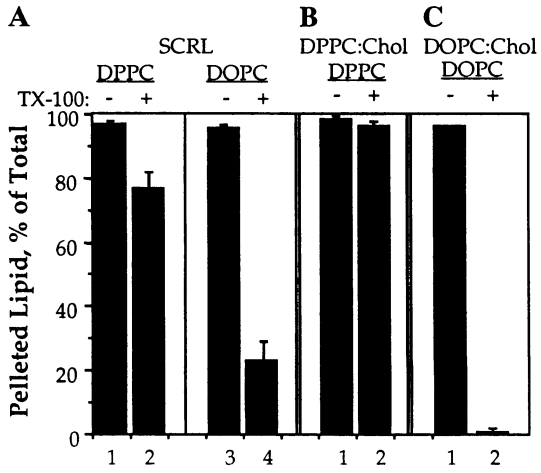

FIG. 2. Solubility of [^{3}H]DPPC and [^{14}C]DOPC in SCRL (*A*) or 2:1 DPPC/Chol (*B*) or DOPC/Chol (*C*) liposomes. Liposomes contained a trace amount (1–2 × 10^4 cpm) of [^{3}H]DPPC (*A*, bars 1 and 2; *B*) or [^{14}C]DOPC (*A*, bars 3 and 4; *C*). Radioactivity in supernatant and pellet fractions was measured after Triton X-100 extraction on ice (*A*, bars 2 and 4; *B* and *C*, bars 2) or incubation without detergent (*A*, bars 1 and 3; *B* and *C*, bars 1) and ultracentrifugation. The percent pelleted lipid [cpm in pellets/(cpm in supernatants + cpm in pellets)] is shown. Number of repetitions of each experiment: *A*, n = 3; *B* and *C*, except *C*, bar 1, n = 2; *C*, bar 1, n = 1.

(19), consistent with the hypothesis that acyl chain saturation and a correspondingly increased resistance to melting correlates with partitioning into detergent-resistant lipid domains.

PLAP, but Not SCRL Lipid, Is Solubilized by Triton X-100 at 37°C. In a previous study (1), PLAP was solubilized from cell membranes by Triton X-100 at 37°C, though not when the extraction was performed on ice. To compare the behavior of PLAP and lipids between SCRL and cell membranes, we repeated the extraction of SCRL by Triton X-100 at 37°C and examined the solubility of PLAP and lipids. PLAP was fully solubilized (data not shown). By contrast, all the lipids in SCRL were solubilized to the same extent whether the extraction was performed on ice or at 37°C. Cell membrane lipids were also incompletely solubilized by Triton X-100 at elevated temperatures (K. Melkonian and D.A.B., unpublished data). Thus, the effects of detergent extraction of PLAP and lipids from SCRL and cell membranes are similar.

SCRL Are Not Fully Fluid and Become Less Fluid After Detergent Extraction. As SCRL contain lipids with unusually high melting temperatures, they should be less fluid than lipids in the liquid crystalline state. To test this idea, we incorporated the fluorescent probe DPH into SCRL and other liposomes and measured fluorescence polarization (Table 2). For comparison, liposomes prepared by sonication and three freeze–thaw cycles were also assayed at 24°C (Table 2). Values varied somewhat between preparations, and between measurements made at 15, 24, or 37°C. However, the same pattern was observed in all cases, so only the results obtained for liposomes prepared by dialysis and measured at 37°C are discussed.

In DPPC liposomes, lipids are present in the nonfluid gel phase. The high observed value of 0.381 for fluorescence polarization of DPH incorporated into these liposomes is as expected for gel-phase liposomes. DOPC and bPC liposomes are in the fluid liquid crystalline state, and, as expected, fluorescence polarization of DPH in these liposomes had a low value, about 0.1. At the temperatures and compositions we used, DPPC/Chol liposomes are in the liquid ordered (l_o) phase (20), a phase intermediate between gel and liquid crystalline, and gave an intermediate polarization of 0.281.

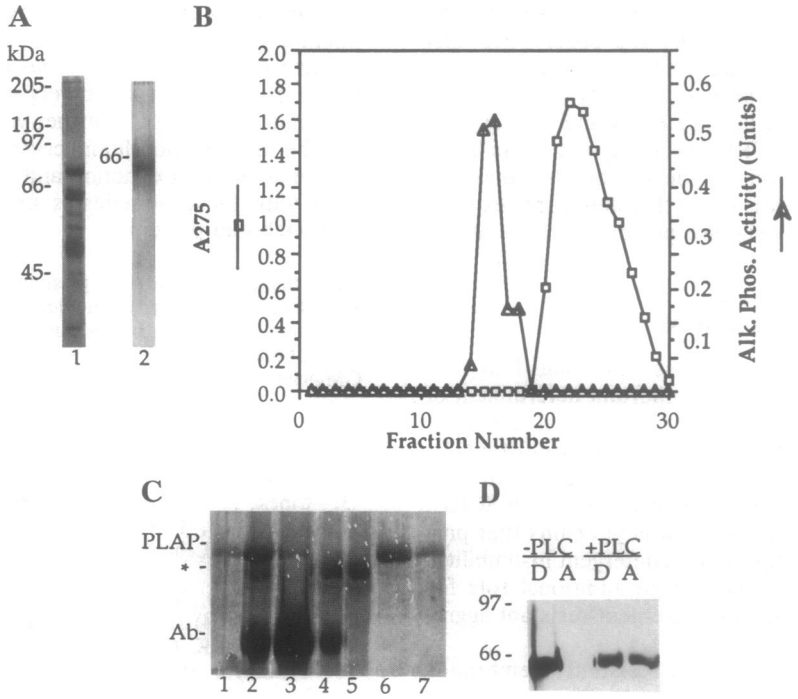

FIG. 3. Purification of PLAP. PLAP from Sigma, partially pure as determined by silver staining (*A*, lane 1), was subjected to Triton X-114 phase separation. The detergent phase was incubated with excess CHAPS and loaded on a Sephacryl S200 column. Fractions (0.5 ml) were collected and analyzed for the presence of Triton X-114 by measuring absorbance at 275 nm (*B*, squares). Alkaline phosphatase activity was assayed in fractions 10–19 (*B*, triangles) by using a Sigma kit. Fractions 14–18 were pooled and subjected to SDS/PAGE and detection of protein by silver staining (*A*, lane 2). The single band on the silver-stained gel in *A* was PLAP, as it could be depleted from the solution by immunoprecipitation. As shown in *C*: lanes 1 and 7; 50 ng of purified PLAP; lane 2, PLAP immunoprecipitated from the purified preparation (750 ng); lane 3, TCA precipitation of protein in the supernatant after immunoprecipitation; lane 4, proteins eluted from *Staphylococcus aureus* cells after incubation with anti-PLAP antibody alone, without addition of PLAP; lane 5, proteins eluted from *S. aureus* cells after incubation with 750 ng of PLAP protein, without antibody; lane 6, TCA precipitation of protein in the supernatant after the no-antibody incubation. Star indicates a protein migrating slightly faster than PLAP, originating from the *S. aureus* cells. Ab, antibody heavy chain. PLAP was shown to be GPI-anchored by incubation with PI-PLC (*D*). To remove CHAPS, 100 μl of precondensed Triton X-114 was added to 1.5 μg of CHAPS-solubilized PLAP in 300 μl and dialyzed overnight against PBS. Phase separation was performed and the aqueous phase was discarded. Fresh buffer with (+PLC) or without (−PLC) PI-PLC was added to proteins in the detergent phase. After incubation at 37°C, phase separation was repeated and proteins were precipitated from detergent (lanes D) and aqueous (lanes A) phases with TCA, subjected to SDS/PAGE, and transferred to nitrocellulose. PLAP was detected by Western blotting.

We then measured the fluorescence polarization of DPH incorporated into SCRL, or into SCRL after detergent extraction. A value of 0.270 was found for SCRL without detergent extraction. The fluorescence polarization of DPH incorporated into SCRL after Triton extraction increased to 0.317, consistent with the preferential extraction of the phospholipids, which tend to favor the presence of the liquid crystalline phase. These values are similar to the value of 0.281 found for the DPPC/Chol liposomes and suggest that SCRL are largely present in the l_o state even at 37°C. Alternatively, the lipids could be in the form of a mixture of liquid crystalline, l_o, and/or gel states. Coexistence of these phases has been observed under a variety of conditions (20).

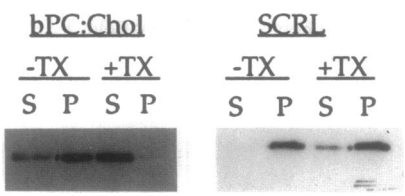

FIG. 4. Solubility of PLAP in 7:1 bPC/Chol liposomes (*Left*) or SCRL (*Right*). Liposomes containing PLAP were subjected to Triton X-100 extraction on ice (+TX) or incubation without detergent (−TX) and ultracentrifugation. PLAP was precipitated with TCA from supernatant (lanes S) and pellet (lanes P) fractions, analyzed by SDS/PAGE and Western blotting, and quantitated by scanning densitometry.

Lipid State Determines Extractability. DPH polarization also suggests a strong correlation between Triton extractability and lipid phase. Previous studies have shown that gel-phase lipids are insoluble in Triton X-100, whereas in the liquid crystalline state lipids are soluble (21). We found that DPPC/Chol liposomes were also insoluble in the detergent. Therefore, it appears that the l_o state is also Triton X-100

Table 2. Polarization of DPH fluorescence in liposomes of various compositions

Liposome lipid composition	Dialysis			Sonication
	37°C	24°C	15°C	24°C
DPPC	0.381	0.405	0.410	0.443
bPC	0.115	0.123	0.163	—
DOPC	0.095	0.123	0.156	0.131
DPPC/Chol, 2:1	0.281	0.291	0.306	0.418
DOPC/Chol, 2:1	0.156	0.190	0.225	0.221
bPC/CB/SM/Chol, 2:1:1:2	0.270	0.296	0.313	0.369
bPC/CB/SM/Chol 2:1:1:2 (extr)*	0.317	0.329	0.333	0.404

The fluorescence polarization of DPH in liposomes was measured at the indicated temperatures. Values shown are from one representative experiment, using liposomes prepared by dialysis or sonication as indicated.

*Liposomes were extracted with Triton X-100 before addition of DPH.

insoluble. [That insolubility is due to lipid state and not the presence of cholesterol is shown by the observation that DOPC/Chol liposomes, which gave a low polarization value of 0.156 and are therefore probably largely in the liquid crystalline state, were solubilized (Table 1 and Fig. 2).] We conclude that the lipids in SCRL are largely not in the liquid crystalline state and that this is the reason that they are insoluble in Triton X-100. It is likely that detergent-resistant membranes isolated from cells are present in this state as well.

DISCUSSION

We have shown that SCRL are less fluid than bulk cell membranes. This appears to be an important determinant of detergent insolubility. It has been proposed that hydrogen bonds between glycosphingolipids cause them to cluster (11). However, DOPC and DPPC have the same capacity to form hydrogen bonds but were extracted differently from SCRL. This shows that interactions between acyl chains that promote a nonfluid state are important in detergent insolubility. However, our data do not rule out an additional role for hydrogen bonds in clustering into detergent-resistant aggregates.

PLAP associates with detergent-resistant membranes when SCRL are extracted with Triton on ice, but not at 37°C. This does not necessarily mean that PLAP is present in liquid crystalline membranes at 37°C. Detergents can perturb the phase equilibrium and solubilize lipids when they are just below their transition temperature, as has been seen for DPPC in its gel state (21). Therefore, a molecule in the gel or l_o state could be solubilized if it is near its melting temperature. Our result suggests that the "effective melting temperature" of the acyl chains of PLAP is lower than that of the sphingolipid/cholesterol mixture in SCRL, but that it could be slightly above 37°C.

As glycosphingolipids are concentrated in caveolae (22, 23) and caveolae can be isolated by detergent extraction (7, 8), the caveolar membrane may exist in a non-liquid crystalline state. In epithelial cells, which contain an unusually high amount of glycosphingolipid in their apical membranes (11), domains of non-liquid crystalline membrane may not be confined to caveolae but may also be present in the surrounding plasma membrane. This might explain why most of the detergent-resistant membranes isolated from epithelial cells do not have the appearance of caveolae (1, 2), while many of those isolated from fibroblasts (2) and smooth muscle cells (8) do.

The ability to associate with nonfluid membranes in cells may be an important general function of GPI anchors. However, GPI-anchored proteins in cells are reported to cluster in caveolae only after crosslinking with antibody (24). Detergent extraction, like antibody crosslinking, may cause redistribution of GPI-anchored proteins into caveolae. Possibly, small clusters of lipids and GPI-anchored proteins in a non-liquid crystalline state move freely in membranes, and enter or are retained in caveolae only when larger aggregates are formed by crosslinking or by extraction of surrounding liquid crystalline membrane.

Our work has provided a reasonable physical explanation for the puzzling fact that detergent-resistant membranes can be isolated from cells. Further work is needed to determine whether discrete non-liquid crystalline domains exist in cells before detergent extraction and whether the ability to associate with these domains is important to the function of GPI-anchored proteins.

We thank J. Volwerk for plasmid pIC, G. Arreaza for purifying PI-PLC, and S. Mayor, K. Fiedler, K. Simons, and R. Anderson for communicating results before publication. This work was supported by National Institutes of Health Grants GM47897 (D.B.) and GM48596 (E.L.).

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