J. Membrane Biol. 191, 215–221 (2003)
DOI: 10.1007/s00232-002-1056-y

The Journal of
Membrane
Biology

Role of GPI-anchored Enzyme in Liposome Detergent-Resistance

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Received: 8 July 2002/Revised: 18 October 2002

Abstract. In this work, we investigated the role of a glycosylphosphatidylinositol (GPI)-anchored protein, the alkaline phosphatase, on the solubilization of detergent-resistant liposomes. In vivo, GPI-anchored proteins are clustered into sphingolipid- and cholesterol-rich membrane domains and this peculiar composition provides cold-detergent-insolubility. To better understand the mechanisms involved in the clustering of these subdomain components, we built a model, namely sphingolipid- and cholesterol-rich liposomes. We show the cold-Triton X-100 resistance of liposomes before and after insertion of GPI-anchored enzyme. When the amount of incorporated enzyme varied, significant changes in membrane stability occurred. Low protein contents into liposomes increased detergent insolubility, whereas high amounts decreased it. Furthermore, significant differences in the detergent-resistance of each lipid were exhibited between liposomes and proteoliposomes. Thus, the enzyme insertion led to a dramatic decrease of cholesterol solubilization, in line with the existence of cholesterol/GPI interactions.

Effect of temperature on detergent resistance was also investigated. Liposome solubilization increased with temperature up to a threshold value of 40/45°C. This was also the temperature at which a phase transition of liposome membrane occurred, as evidenced by Laurdan fluorescence. Although the GPI-anchored enzyme insertion modified membrane stability, no change was observed on phase transition. Our work highlights the importance of GPI-anchored proteins in the structure of sphingolipid- and cholesterol-rich membrane domains, in the detergent-insolubility of these peculiar domains, as well as in interaction of GPI proteins with cholesterol.

Key words: Liposomes — Detergent-resistance — Cholesterol — Alkaline phosphatase — GPI — Membrane phase transition

Introduction

Evidence is now accumulating that the plasma membrane is organized in different lipid and protein subdomains (Mouritsen & Jørgensen, 1997). Thus, GPI-anchored proteins are clustered into sphingolipid (SL)- and cholesterol (Chol)-rich membrane domains, also called rafts or detergent-resistant membranes (DRMs) (Harder & Simons, 1997; Friedrichson & Kurzchalia, 1998; Hooper, 1999). GPI-anchored proteins are functionally diverse proteins, including hydrolytic enzymes, protozoan surface proteins, adhesion proteins, surface antigens, receptors and prion protein (Hooper, 1992). In addition to affording the protein association with the membrane, the GPI lipid tail presents important biological functions, especially in signal transduction, membrane addressing and recognition processes (Nosjean, Briolay & Roux, 1997). This anchor confers more lateral mobility to proteins and takes less space than their transmembrane domain, allowing the proteins to pack tightly. Lastly, anchor hydrolysis by specific phospholipases induces the selective release of these cell-surface proteins into the extracellular medium. This process may modulate the structure and the function of GPI-anchored proteins (Hooper, 1992; Nosjean et al., 1997).

Rafts, where GPI-anchored proteins are clustered, are postulated to act as moving relay stations

Abbreviations: BIAP, alkaline phosphatase from bovine intestinal mucosa; Chol, cholesterol; DRMs, detergent-resistant membranes; GC, galactocerebrosides; g_0, gel-ordered; GPI, glycosylphosphatidylinositol; l_d, liquid-disordered; l_o, liquid-ordered; OctGlc, *n*-octyl β-D-glucoside; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PL, phospholipids; SL, sphingolipids; SM, sphingomyelin; T_m, melting temperature.

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in membrane trafficking and signal transduction (Harder & Simons, 1997; Rietveld & Simons, 1998; London & Brown, 2000). These membrane domains can be isolated because of their insolubility in cold non-ionic detergents, such as Triton X-100 (Brown & Rose, 1992; Sargiacomo et al., 1993; Harder & Simons, 1997; London & Brown, 2000). This resistance was also seen in reconstituted membrane systems enriched in SL and Chol (Schroeder, London & Brown, 1994). The SL-Chol mixtures are characterized by their highly ordered and densely packed lipid environment for sufficiently high Chol molar ratio (Brown, 1998). The formation of a liquid-ordered (l_o) phase in these mixtures may induce the detergent-insolubility in artificial lipid vesicles (Sankaram & Thompson, 1990; Schroeder et al., 1994; Ahmed, Brown & London, 1997; Schroeder et al., 1998). It is therefore, likely that phase separation might be the key for the formation of insoluble lipid rafts also in biological membranes (Brown & London, 2000; Rietveld & Simons, 1998).

Paradoxically, all the studies available on this subject do not investigate the role of GPI-anchored proteins on detergent-resistance and phase behavior of biological and artificial membranes (Schroeder et al., 1994; Reid-Taylor, Chu & Sharom, 1999). In order to better understand the role of GPI-anchored proteins on membrane detergent-resistance, we attempted to build a model of these DRMs. We prepared liposomes enriched in Chol, sphingomyelin and cerebrosides, with or without GPI-anchored protein insertion, namely, the alkaline phosphatase from bovine intestinal mucosa (BIAP). We determined the detergent resistance of these liposomes and proteoliposomes, and we demonstrated the dependence of detergent-resistance on GPI-protein insertion into biomimetic membranes.

Materials and Methods

Phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SM), galactocerebroside (GC), cholesterol (Chol), *n*-octyl β-D-glucoside (OctGlc) were purchased from Sigma Chemicals. Dipalmitoyl-phosphatidyl-[*N*-methyl-^{3}H]-choline ([^{3}H]-PC) and [4-^{14}C]-cholesterol ([^{14}C]-Chol) were purchased from NEN Products. Laurdan (6-dodecanoyl-2-dimethylaminonaphthalene) was purchased from Molecular Probes.

Liposome Preparation

Large unilamellar vesicles were prepared by the freeze-thaw and extrusion procedure, as previously described (Morandat, Bortolato & Roux, 2002). A typical preparation contained 10 mg of lipids solubilized in 1 ml of chloroform. The organic solvent was removed by N_2 flow under atmospheric pressure. The lipid film was then dispersed in 500 μl 10 mM Tris-HCl, 150 mM NaCl, pH 8.5 buffer (TBS) and shaken for 15 min. The hydrated lipid dispersion was exposed to six freeze-thaw cycles (−180°C/+25°C for PC liposomes or −180°C/+45°C for SL-containing liposomes) and passed 39 times through a polycarbonate membrane (0.2 μm) with a Mini-Extruder apparatus (Avanti Polar Lipids). The total lipid concentration of the resulting liposome suspension was 20 mg/ml. Several kinds of liposomes were prepared: PC liposomes, GS liposomes (PC/PE/GC/SM, 1:1:1:1) and GSC liposomes (PC/PE/GC/SM/Chol, 1:1:1:1:2). We used [^{3}H]-PC to label all the liposomes, [^{14}C]-Chol provided additional labeling to Chol-containing vesicles, in proportion of 0.1 μCi/ml for each one.

Preparation of BIAP Proteoliposomes

Proteoliposomes were reconstituted according to Morandat (Morandat et al., 2002). Briefly, alkaline phosphatase (E.C. 3.1.3.1) was purified from mucosa of fresh bovine intestine. Incorporation of BIAP was performed by incubation of 1 mg/ml liposomes with 16 mM OctGlc for 15 min at 25°C, followed by addition of enzyme. The detergent was then removed by hydrophobic adsorption on Amberlite XAD-2 resin at 4°C. Proteoliposomes were separated from free remaining BIAP by ultracentrifugation in 2.5–30% (w/v) sucrose gradient, at 160,000 × g, 2 hr at 4°C.

Solubilization of Liposomes and BIAP Proteoliposomes

Increasing concentrations of Triton X-100 were added to liposome or proteoliposome mixtures suspended in TBS and incubated at working temperature for 30 min. For each sample, final lipid concentration was 1 mg/ml and the Triton X-100 percentage was were expressed in v/v. We used the inability of lipid or protein to pellet during centrifugation (200,000 × *g* for 2 hr at 4°C) as a criterion of solubilization, as used by several authors (Schroeder *et al.*, 1994; Parkin, Turner & Hooper, 2001). [^{3}H]-PC and/or [^{14}C]-Chol radioactivities were counted in pellets and supernatants. For proteoliposomes, BIAP activities were also measured. The solubilization rate corresponds to the ratio between the [^{3}H]-PC supernatant content and the [^{3}H]-PC total content (*idem* for [^{14}C]-Chol content and BIAP activity). Each experiment was repeated five times and standard deviations (SD) were determined.

Enzyme Activity

Aliquots of enzyme solutions were taken and diluted in 10 mM glycine-NaOH buffer, pH 10.4, containing *p*-nitrophenyl phosphate as enzyme substrate. Activities were measured spectrophotometrically at 37°C, according to Bortolato (Bortolato, Besson & Roux, 2002).

Laurdan Fluorescence

To detect a possible phase transition of GSC liposomes and proteoliposomes, we used Laurdan, an amphiphilic fluorescent probe whose emission depends strongly on the physical state of the membrane. At temperatures below the lipid phase transition (gel state), the emission maximum is in the blue region of the spectrum. At temperatures above the lipid phase transition (liquid state) the emission maximum is red-shifted. The fluorescence parameter known as the generalized polarization (*GP*) relates these spectral changes quantitatively at each temperature, by taking into account the relative fluorescence intensities of the blue and red edge regions of the emission spectra. The generalized polarization (*GP*) is given by:

$$GP = (I_B - I_R)/(I_B + I_R) \quad (1)$$

where I_B and I_R are the fluorescence intensities at the blue and red edges of the emission spectrum, respectively (Parasassi et al., 1991).

This probe has been used to study phase fluctuations and main T_m of vesicles containing various lipids, i.e., PL, Chol and SL (Parasassi et al., 1991; Bagatolli *et al.*, 1997; Bagatolli, Gratton & Fidelio, 1998).

Fluorescence spectra were recorded with a Hitachi F 4500 fluorimeter (150W Xe). The excitation and emission bandwidths were 2.5 and 5 nm, respectively. Laurdan (1 μM) was added to liposomes and proteoliposomes (110 μM). Samples were heated above the main T_m prior to fluorescence measurements. Next, they were cooled to 10°C and the temperature was then raised stepwise from 5 to 95°C. The temperature was controlled by a circulating bath and measured directly in the sample cuvette by a digital thermometer. The spectrum was recorded 5 min after the selected temperature was reached. Laurdan emission spectra were recorded using a 360 nm excitation wavelength. Each spectrum was deduced from three scan averages and the blank spectrum (sample without probe). Each experiment was repeated three times.

Results and Discussion

Effect of BIAP Insertion on Cold Triton X-100 Resistance of GSC Liposomes

BIAP was incorporated by detergent into PC, GS (PC/PE/GC/SM, 1:1:1:1) and GSC (PC/PE/GC/SM/Chol, 1:1:1:1:2) liposomes at a protein/lipid ratio of 10 (μg BIAP/mg liposomes—i.e., μg protein/mg lipid). Proteoliposomes were then subjected to Triton X-100 extraction at 4°C. For reference, identical experiments were performed on liposomes without protein. As control experiments, Triton X-100 extraction at 4°C was also performed on liposomes subjected to the reconstitution procedure but without protein.

For PC proteoliposomes (Fig. 1*A*), lipids were largely solubilized by 0.2% Triton X-100, as for PC liposomes (Fig. 1*B*). Total extraction of BIAP was obtained for 0.5% Triton X-100. Inversely, BIAP insertion into GS and GSC liposomes led to significant modifications of lipid detergent-resistance. In GS proteoliposomes, the lipid solubilization rates were dramatically enhanced, to 80% at 2% Triton X-100 (Fig. 1*C*) compared with 50% for GS liposomes (Fig. 1*D*). In contrast, in GSC proteoliposomes (Fig. 1*E*) these rates were lower than in GSC liposomes (Fig. 1*F*). Moreover, Chol solubilization rates displayed a reversal between GSC liposomes and proteoliposomes: they were the highest in liposomes and the lowest in proteoliposomes. Thus, at 12% Triton X-100, only 45% of Chol and 60% of PC were solubilized in GSC proteoliposomes, compared with, respectively, 87% and 65% in GSC liposomes. As for BIAP solubilization, while consistent with those of lipids, rates were always lower for the three kinds of proteoliposomes. The lowest ones were displayed in GSC proteoliposomes, with 30% at 12% Triton X-100. Lastly, the control experiments demonstrated that the liposome incubation with OctGlc in the incorporation procedure does not affect the lipid solubilization behavior, as the same results were obtained with and without OctGlc treatment.

To quantitate the relationship between the amount of BIAP inserted and proteoliposome detergent-resistance, solubilization rates for 2% Triton X-100 were plotted versus BIAP/lipid ratios (μg/mg) in GSC liposomes (Fig. 2). Insertion of low amounts of BIAP (up to a BIAP/lipid ratio of about 80) increased the lipid detergent-resistance (i.e., lowered the solubilization rate), whereas resistance decreased when this ratio reached about 100. As for BIAP, its own solubilization rate increased, to a lesser degree, with protein/lipid ratio. Similar results were obtained with 6 and 8% Triton X-100 (*data not shown*).

These studies at 4°C demonstrate that SL- and Chol-rich liposomes with or without inserted BIAP (liposome ± BIAP) behaved like DRMs since they were not fully solubilized by 20% Triton X-100. In artificial lipid vesicles, the presence of ordered membrane domains provides detergent resistance (Schroeder et al., 1994, 1998; Ahmed et al., 1997). PC melts below 0°C therefore PC liposomes ± BIAP were in liquid-disordered (l_d) phase at 4°C and thus were easily solubilized. In GS liposomes ± BIAP, the high T_m and the high ratio of SL and saturated PL might induce these lipids to form a separate gel-ordered (g_o) phase of both at 4°C, a phase which promotes detergent resistance, as shown previously (Schroeder et al., 1994; Ahmed et al., 1997). But for Chol-containing liposomes, at a threshold sterol concentration, phase separation occurs with the formation of the phase that promotes detergent-resistance phase, i.e., the liquid-ordered (l_o) phase, in mixtures of lipids with highly saturated acyl chains, such as GC, SM and also saturated PC (Sankaram & Thompson, 1990; Ahmed et al., 1997). Because the Chol fills the voids between the PL acyl chains and interacts with them, the packing density of the bilayer increases. But when SL are present, their stronger lateral cohesion, induced by hydrogen bonds between the sphingosine backbones and/or sugar head groups, provides the dramatic increase of packing density that leads to l_o phase formation. Our data thus show that at 4°C, for similar SL mole content, the lipid solubilization was higher in Chol-lacking liposomes ± BIAP (i.e., GS liposomes ± BIAP) than in Chol-containing liposomes ± BIAP (i.e., GSC liposomes ± BIAP). The lipid detergent-insolubility was enhanced by the presence of Chol and might be induced by the formation of the l_o phase in GSC liposomes, whereas GS liposomes contained lipids only in the l_d or g_o phase. Similar observations were made with SL/Chol-rich liposomes (Schroeder et al., 1998).

After incorporation into liposomes, BIAP was solubilized at 4°C in a way similar to that of lipids. In PC proteoliposomes, BIAP was easily solubilized to the same degree as PC. In contrast, in GS and GSC proteoliposomes, GPI-anchored BIAP might associ-

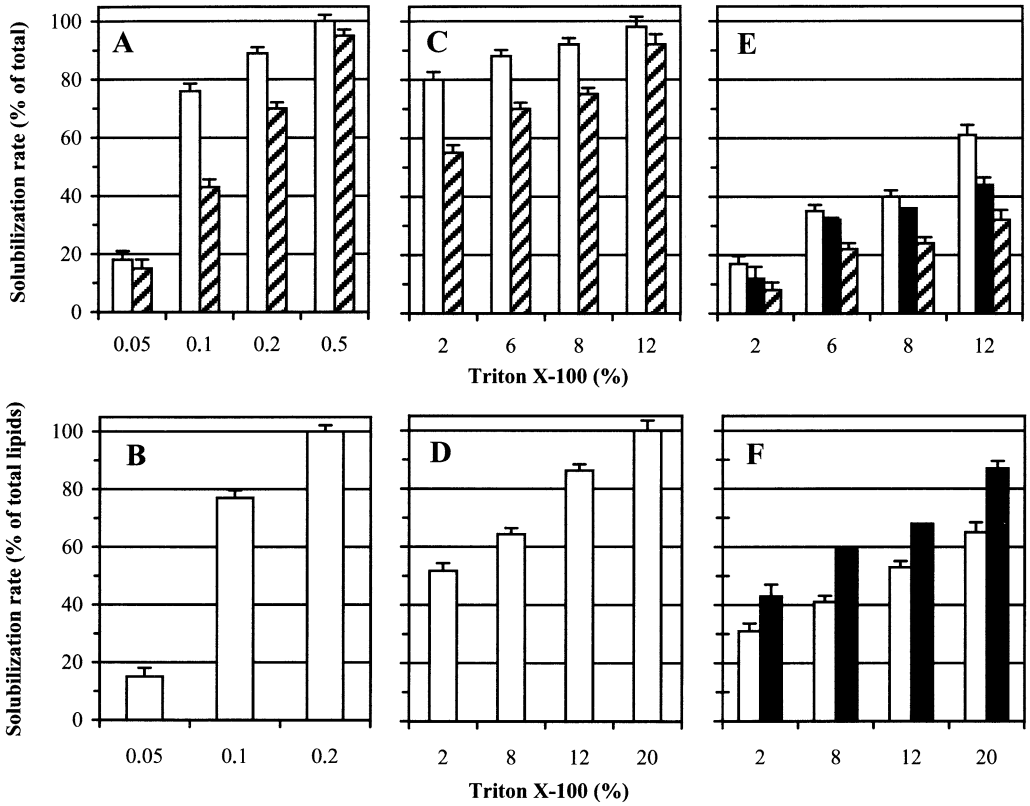

Fig. 1. Solubilization of liposomes and proteoliposomes by Triton X-100 at 4°C. Liposomes or proteoliposomes were solubilized on ice by Triton X-100. The lipid concentration was 1 mg/ml. Insoluble material was pelleted by ultracentrifugation. The radioactivity or BIAP activity was measured in supernatant and pellet fractions: [^{3}H]-PC for all (proteo)liposomes, [^{14}C]-Chol for GSC (proteo)liposomes and enzyme activity for all proteoliposomes. The fraction of the total that was soluble is plotted versus the Triton X-100 concentration used. The values are mean ± SD. Solubilization rates of: [^{3}H]-PC, *white bars*; [^{14}C]-Chol, *black bars*; BIAP activity, *striped bars*. Solubilization of PC proteoliposomes (*A*) and PC liposomes (*B*); solubilization of GS proteoliposomes (*C*) and GS liposomes (*D*); solubilization of GSC proteoliposomes (*E*) and GSC liposomes (*F*). For proteoliposomes, the protein/lipid ratio was 10 μg/mg; GS for PC/PE/GC/SM (1:1:1:1) and GSC for PC/PE/GC/SM/Chol (1:1:1:1:2).

ate directly with insoluble lipids, i.e., with SL. Interactions between GPI and lipids were sufficient for cold-detergent insolubility of the protein, as shown previously for placental alkaline phosphatase (Schroeder et al., 1994). Fatty acyl chains of GPI anchors are more saturated than those found in membrane PL (McConville & Ferguson, 1993), consistent with the hypothesis that acyl-chain saturation and a corresponding increased resistance to melting correlate with partitioning into DRMs.

At low protein contents, lipids were more resistant to Triton X-100 action, whereas higher protein levels enhanced the solubilization rates of lipids and protein. In the reconstitution of Thy-1 into lipid vesicles of dimyristoylphosphatidylcholine and GC, at low protein:lipid ratios, the protein preferentially sequestered GC to form enriched microdomains. At higher ratios, Thy-1 could alter its conformation in response to steric hindrance within these domains, such that its interaction with the bilayer surface is reduced (Reid-Taylor et al., 1999). In the same way, BIAP, at low contents, could interact closely with lipids and might increase the packing of the bilayer, and thus increase detergent resistance, whereas at high contents, steric hindrance could induce small bilayer perturbations — e.g., less packing of acyl chains — which could reduce detergent resistance of both lipids and BIAP. The increase in solubilization of the enzyme at the highest protein:lipid ratios may also originate from the perturbation caused by the coexistence of different phases. Because of the GPI insertion into the bilayer, the ratios between the different lipids were modified, and consequently phase miscibility could occur, enhancing solubilization. In another hypothesis, the insertion would modify the elasticity of the membrane, since this parameter seems to be involved in establishing the structural features of microdomains (Li et al., 2001).

The reversal observed in Chol solubilization rates after BIAP insertion into GSC liposomes led to the hypothesis of direct interactions between Chol and the BIAP anchor. This idea is strengthened by the Chol-

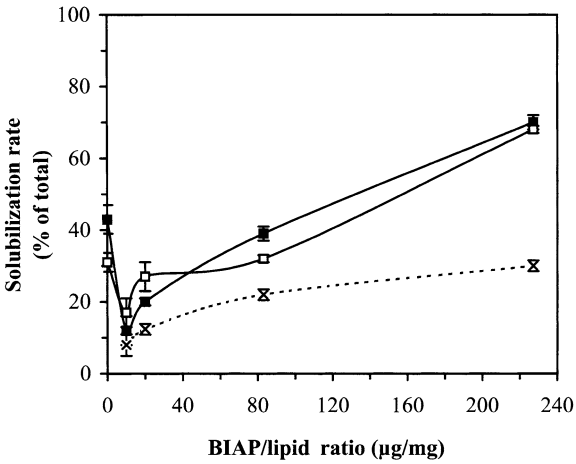

Fig. 2. Effect of protein/lipid ratio on lipid and BIAP solubilization rates of GSC proteoliposomes at 4°C. The lipid concentration was 1 mg/ml. Proteoliposomes containing different amounts of BIAP were solubilized on ice by 2% Triton X-100. Insoluble lipids and protein were pelleted by ultracentrifugation, and [^{3}H]-PC (□), [^{14}C]-Chol (■) and BIAP activity (×) were measured in supernatant and pellet fractions. Solubilization rates are plotted as a function of BIAP/lipid ratio (µg BIAP/mg liposomes). Each point and bar show mean ± SD.

dependent insertion of BIAP into liposomes (Morandat et al., 2002), which is also seen by atomic force microscopy (Milhiet et al., 2002). The existence of GPI/Chol interactions may explain the Chol dependence of the detergent insolubility of GPI-anchored proteins in both cellular membranes and artificial vesicles (Hanada et al., 1995; Schroeder et al., 1998; Scheiffele et al., 1999; Lipardi, Nitsch & Zurzolo, 2000). Moreover, as a raft component, Chol has a functional significance in membrane trafficking (Keller & Simons, 1998; Hansen et al., 2000). Chol is also prerequisite for signal transduction via GPI-anchored proteins in T cells (Stulnig et al., 1997). In caveolae, the clustering of GPI-anchored protein is controlled by lipid-lipid interactions between the fatty acyl chains of the bilayer, and Chol plays an essential role in this phenomenon (Rothberg et al., 1990). In the same way, in living cells, GPI-anchored proteins are clustered in rafts and that clustering is dependent on the level of Chol in the cell (Friedrichson & Kurzchalia, 1998).

Effect of Temperature on Detergent Resistance

To study the behavior of lipids at physiological temperature, we repeated the extraction with various concentrations of Triton X-100 at 38°C. Solubilization rates were determined for PC, GS and GSC liposomes. The solubilization rates of control PC liposomes exhibited no change at 38°C compared to 4°C (*data not shown*). In contrast, temperature increase induced total solubilization of GSC liposomes by 1% Triton X-100 (Fig. 3*A*); this was true also for GS liposomes (*data not shown*).

To quantitate the temperature dependence of the solubilization, GSC liposomes were incubated with a fixed concentration of Triton X-100 (0.15%) at various temperatures between 4 and 70°C. Solubilization rates were determined as described before and are shown in Fig. 3*B*. Lipid solubilization increased with temperature, reaching a plateau at 40°C.

In the case of PC liposomes, solubilization rates were identical at 4 and 38°C because PC was in the liquid-disordered (l_d) phase at both temperatures. In the case of GS liposomes, when the temperature was raised above T_m, GS, as expected, was then in the l_d phase and so was fully solubilized. As for GSC liposomes, the lipid solubilization rates increased with temperature up to a threshold value. Although Chol promoted phase separation, this phenomenon is temperature dependent (Ahmed *et al.*, 1997; Sankaram & Thompson, 1990), as observed here. For lipid solubilization, the rates exhibit an increase similar to those for GSC liposomes at 38°C (*data not shown*)

When BIAP was inserted into GSC liposomes, detergent extraction at 38°C improved solubilization rates dramatically for lipids as well as for BIAP, whatever the BIAP/lipid ratio (*data not shown*). The enzyme was fully solubilized with 1% Triton X-100. As for lipid, no significant change was observed in solubilization rates in respect to liposomes.

Phase Transition in GSC Liposomes and Proteoliposomes

As detergent-resistance is linked to the membranous phase state, phase transitions of GSC liposomes and proteoliposomes were measured using the fluorescence emission of Laurdan. Laurdan is sensitive to the phase transition of GSC liposomes and proteoliposomes, as previously described for other kinds of vesicles (Parasassi et al., 1991; Bagatolli et al., 1997; Bagatolli et al., 1998). Emission spectra of GSC liposomes and proteoliposomes were recorded at temperatures from 5°C to 95°C. They showed a continuous shift to longer wavelengths. Thus, the emission maximum centered at 435 nm at 5°C was red-shifted to 490 nm at 95°C (*data not shown*).

To estimate the phase transition temperature, the generalized polarization (*GP*) was calculated, as described in Methods. The Laurdan *GP*, plotted as a function of temperature (Fig. 4), showed that the value decreased with increasing temperature for liposomes as well as for proteoliposomes. Sigmoid relationships were obtained, showing the sensitivity of Laurdan *GP* to the phase transition of the GSC liposomes and proteoliposomes, which occured for both at about 45°C. Studies on binary mixtures such as PL/Chol and SM/Chol vesicles suggest that the two liquid phases, l_o and l_d, coexist at certain Chol mole ratios. But at a critical temperature above the main T_m of the pure lipid associated with Chol, the l_o

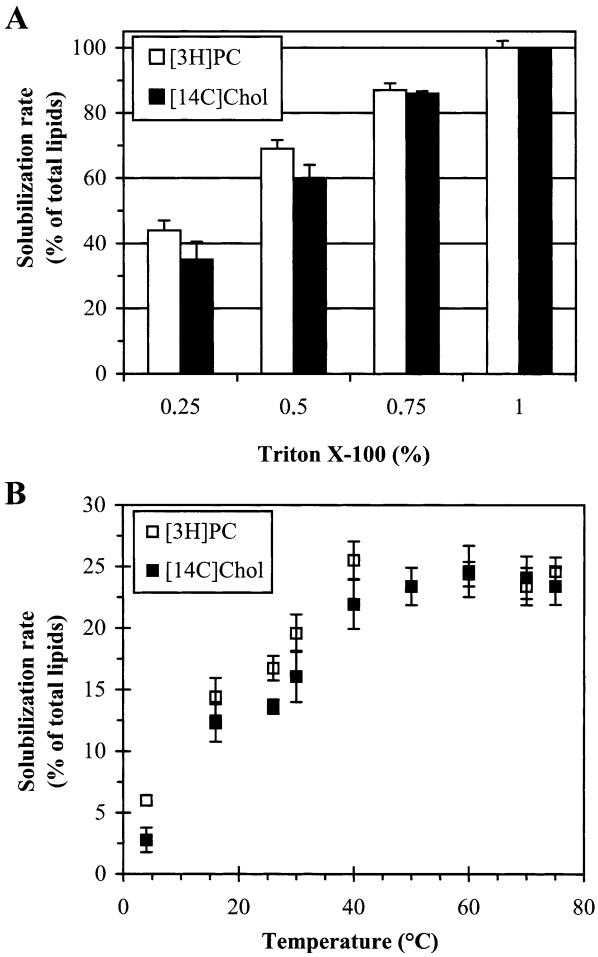

Fig. 3. Effect of temperature on solubilization of GSC liposomes by Triton X-100. The lipid concentration was 1 mg/ml. Insoluble lipids were pelleted by ultracentrifugation, and the radioactivity in supernatant and pellet fractions was measured for [^{3}H]-PC and [^{14}C]-Chol. The values are mean ± SD. (*A*) GSC liposomes were solubilized at 38°C by Triton X-100. The fraction of the total that was soluble is represented as a function of the concentration of Triton X-100 used. (*B*) GSC liposomes were solubilized at different temperatures by 0.15% Triton X-100. Solubilization rates are plotted as a function of temperature.

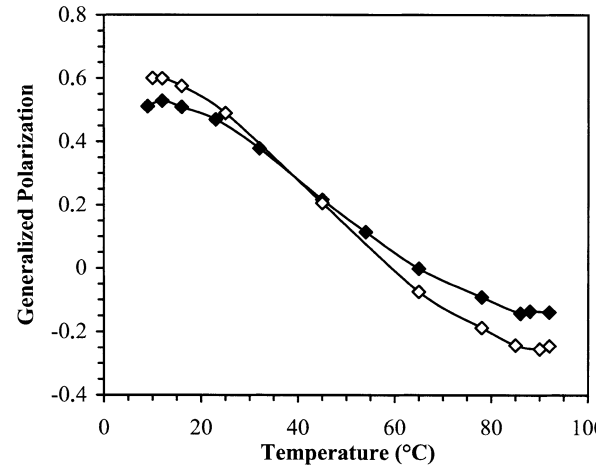

Fig. 4. Effect of temperature on Laurdan *GP* values of GSC liposomes and GSC proteoliposomes. Concentrations of Laurdan and liposomes were 1 μM and 110 μM in TBS, respectively. For proteoliposomes, lipid concentration was 110 μM and BIAP/lipid ratio was 230 μg/mg. The excitation wavelength was set at 360 nm. The fluorescence intensities were measured at emission wavelenghts of 435 and 490 nm. The *GP* values are plotted as a function of temperature. ◇ GSC liposomes; ◆ GSC proteoliposomes.

and l_d phases become miscible (Sankaram & Thompson, 1990). It should be cautioned here that the phase behavior of ternary and higher-order mixtures is more complex than that of binary mixtures. It is likely that in GSC liposomes a phase transition occurred at about 45°C, correlated with l_d/l_o phase coalescence. This interpretation is in good agreement with the effect of temperature on solubilization rates of GSC liposomes shown on Fig. 3*B*. The linear dependence observed from 4°C to 40°C, may reflect the progressive disappearance of the l_o phase. At a threshold temperature, i.e., at about 40/45°C, solubilization rates became constant, presumably because phase coalescence has occurred, as suggested by the fluorescence data.

BIAP insertion into GSC liposomes at a high protein/lipid ratio did not affect the phase transition, as shown on Fig. 4. The same results were obtained at low protein/lipid ratios (*data not shown*). Thus, the modifications induced by GPI insertion into the membrane were sufficient to modify the detergent resistance, but they were too weak to promote a change in phase transition, which is a global parameter.

In conclusion, our work highlights the role of GPI-anchored proteins in the complex the detergent resistance of these membranes, as well as in the peculiar interactions with cholesterol, a key raft component. The determination of the interactions between proteins and lipids in rafts will become important in the next years, as rafts play also a central role in the metabolism of prion protein as well as in the membrane budding of several viruses (Naslavsky *et al.*, 1997; Scheiffele *et al.*, 1999; Nguyen & Hildreth, 2000). The membrane model we described here can now be used to determine the role of membrane lipid composition and its phase state in enzyme activity and therefore a possible role in regulation of the enzyme.

We thank Dr. F. Besson for her critical and helpful reading of the manuscript. We thank also Pr. C. Vial for providing us access to a Hitachi F 4500 fluorimeter and S. Jacquard for technical assistance. This work was supported by the Centre National de la Recherche Scientifique.

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