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Biochimica et Biophysica Acta 1328 (1997) 227–236

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Distinct interactions among GPI-anchored, transmembrane and membrane associated intracellular proteins, and sphingolipids in lymphocyte and endothelial cell plasma membranes

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Received 14 February 1997; revised 15 April 1997; accepted 21 April 1997

Abstract

Glycosylphosphatidylinositol (GPI)-anchored glycoproteins are enriched in sphingolipid-rich plasma membrane domains, which are often isolated as low-density membrane complexes. This association is believed to arise from the interactions between the GPI-acyl chains and sphingolipids, but is not fully understood. In this study, we compared the physical properties of GPI-anchored glycoproteins from a non-polarized (murine T-lymphocyte) and a polarized (human endothelial) cell by equilibrium density gradient centrifugation after extraction by detergents under identical conditions. Unlike those on epithelial cells, the GPI-anchored proteins of lymphocytes (Thy-1 and the heat stable antigen CD24) were enriched in the floating fractions after extraction over a wide range of octylglucoside concentrations. In contrast, the floatability of endothelial GPI-anchored CD59 was markedly diminished, not only by octylglucoside, but also by increasing concentrations of Triton X-100. Distribution of cholera toxin binding ganglioside GM1 in the sucrose gradient fractions closely followed that of the GPI-anchored proteins in both lymphocytes and endothelial cells under most extraction conditions. Analysis of the intracellular acylated molecules revealed that a significant amount of $p56^{lck}$ was always associated with the floating GPI-rich fractions of lymphocytes when extracted by Triton X-100 or octylglucoside at 4°C, while the behaviour of endothelial cell caveolin was comparable to that of CD59. The transmembrane glycoproteins CD45 in lymphocytes and MHC class I antigen in endothelial cells interacted weakly with GPI domains, whereas endothelial CD44 and lymphocyte CD26 displayed a strong association. These results show that: (1) the physical properties of different GPI-anchored proteins may vary significantly; and (2) transmembrane and acylated intracellular proteins could be associated with GPI domains to a variable extent. These differences probably reflect cell type-specific interactions of GPI anchors with the sphingolipid framework of plasma membranes, as well as extracellular interactions of GPI-anchored glycoproteins with neighbouring cell surface molecules. © 1997 Elsevier Science B.V.

Keywords: Glycosylphosphatidylinositol; Plasma membrane domain; Caveola; Sphingolipid; Detergent insolubility

Abbreviations: CT, cholera toxin; ECL, enhanced chemiluminescence; GPI, glycosylphosphatidylinositol; GSL, glycosphingolipids; HSA, heat stable antigen (CD24); TX-100, Triton X-100; OTG, Octylglucoside

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PII S0005-2736(97)00099-0

1. Introduction

A number of mammalian cell surface glycoproteins are modified by a glycosylphosphatidylinositol (GPI) tail that anchors them to the outer leaflet of the plasma membrane [1]. GPI-anchored proteins from a variety of cell types and tissues are poorly solubilized by non-ionic detergents, such as Triton X-100 (TX-100) [2–8], and thus are usually recovered in the form of large multimolecular membrane complexes [9–12] that are also enriched in glycosphingolipids (GSLs) and cholesterol [11,13–16]. In fact, a GPI-anchored protein inserted into sphingolipids and cholesterol-rich lipid vesicles was shown to resist detergent solubilization [17]. Interactions between the acyl chains of GPI anchors and GSLs are thought to occur in the *trans* Golgi network, from where they are transported to the plasma membrane as 'sphingolipid rafts' [11,18]. The rafts accumulate in the apical membrane of polarized cells and can be isolated as light density vesicular complexes by sucrose density gradient centrifugation [11]. Plasma membrane GPI domains have been isolated from both polarized epithelial cells and non-polarized lymphocytes and mast cells [8,10–12,19], as well as neuronal cells [20]. In epithelial cells, these membrane complexes are also enriched in caveolin [21], the major structural component of plasma membrane caveolae [22]. Recent studies on fibroblasts, epithelial and endothelial cells have demonstrated that caveolae and GPI domains are morphologically distinct [23,24], but may be functionally interconnected [25]. The GPI/GSL/caveolin-rich membrane specializations are variously designated as Triton-insoluble floating fractions (TIFFs) [26], detergent-insoluble glycosphingolipid-enriched membrane domains (DIGs) [27], detergent-resistant membrane complexes (DRMs) [28], or simply GPI domains [29]. Even though certain studies have claimed that these domains are detergent-induced artifacts, evidence is accumulating to show that they are true plasma membrane specializations [29–32].

We observed that the floating properties of lymphocyte GPI domains in sucrose gradients markedly differed from those of polarized epithelial cells [11]. We therefore undertook a systematic comparison of non-polarized lymphocyte and polarized endothelial cells' GPI domains. Our results suggest that significant variations exist (1) in the organization of GPI domain components in different cell types and (2) in the way neighbouring glycoproteins associate with GPI domains.

2. Materials and methods

2.1. Reagents and antibodies

Triton X-100 was purchased from Merck (Darmstadt, Germany). OTG was obtained from Alexis (San Diego, CA). Horseradish peroxidase (HRP)-conjugated cholera toxin β-subunit (CT) was from Sigma (Sigma Chemie, Buchs, Switzerland). The enhanced chemiluminescence (ECL) reagent was from Amersham, UK.

Rat mAb against murine Thy-1.2 (mAb 30-H12; ATCC TIB107), CD45 (mAb M1/9.3 · 4HL · 2; ATCC TIB122) and the heat stable antigen (HSA) CD24 (M1/69 · 16.11 · HL; ATCC TIB 125) were obtained from the American Type Culture Collection (Rockville, MD) and used as culture supernatants. Rat anti-murine CD26 hybridoma (H207.773) was a kind gift from Dr. M. Pierres, Centre d'Immunologie, INSERM-CNRS, Marseille. Mouse mAb against human CD59 (MEM-43) and major histocompatibility antigen I (MHC-I) (MEM-149) were kind gifts from Dr. Vaclav Horejsi, Institute of Molecular Genetics, Prague. Rabbit polyclonal antibody against $p56^{lck}$ and HRP-conjugated goat anti-mouse, goat anti-rat and goat anti-rabbit IgG were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Rabbit polyclonal antibody against caveolin was from Transduction Laboratories (Lexington, KY).

2.2. Cell culture, preparation of detergent lysates, and equilibrium density gradient centrifugation

The murine T-lymphoma cell line P1798 obtained from Litton Bionetics (Bethesda, MD) was propagated in syngeneic Balb/c mice as ascites. All the following steps were carried out at 4°C unless indicated otherwise. Cells were washed in PBS twice and once in TKM buffer (50 mM Tris-HCl pH 7.4, 25 mM KCl, 5 mM $MgCl_2$ and 1 mM EGTA). Aliquots of indicated number of cells were lysed directly in 750 μl of lysis buffer (TKM buffer containing the protease inhibitors leupeptin (1 μM), aprotinin (2 μg/ml) and Pefabloc® SC (2 mM) (all from

Boehringer Mannheim), and indicated concentrations of detergent TX-100 or OTG). Lysis was carried out for 20 min at 4 or 37°C as indicated. The lysate was centrifuged in a microfuge at top speed for 3 min to pellet down the nuclei and other sedimentable membrane components, which were solubilized in 0.750 ml of lysis buffer containing 1% SDS by brief sonication. The presence of cell surface and intracellular proteins was assayed in both the postnuclear supernatant (S) and the nuclear pellet (P). To analyse the floatation properties of cell surface components, lysates were subjected to equilibrium density gradient centrifugation as described earlier [33]. Briefly, the whole lysate was adjusted to 40% sucrose by adding equal volume of 80% sucrose, placed at the bottom of Beckman SW41 tubes, overlaid with 6.0 ml of 36% sucrose followed by 3.5 ml of 5% sucrose in TKM buffer, and centrifuged at 38 000 rpm ($250\,000 \times g$) for at least 16 h at 4°C. One-ml fractions were collected from the top excluding the pellet, numbered 1–11 from top to bottom, and stored at −20°C.

Human umbilical vein derived-endothelial cell line ECV304 [34] was obtained from ATCC and maintained in DMEM containing 10% FCS. Confluent cultures in 10-cm diameter Petri dishes were overlaid with 750 μl of lysis buffer (conditions identical to that used for MDCK cells [11]), scraped and incubated for 20 min on ice or at 37°C. Further steps were the same as described above for P1798 cells.

2.3. Detection of cell surface and intracellular proteins

Cell surface and intracellular proteins in detergent extracts and density gradient fractions were detected by dot-immunoassay as described previously [33] or by Western blotting. Briefly, 10 μl of the gradient fractions were dotted onto nitrocellulose filters in 200 ml volume using a dot-blot apparatus (Bio-Rad, Richmond, CA). For Western blotting 20 μl of the gradient fractions were directly solublized in 6× non-reducing SDS–PAGE sample buffer for the detection of cell surface antigens, or in reducing sample buffer for intracellular proteins. Proteins separated using a minigel apparatus (Bio-Rad) were transferred to nitrocellulose filters (Schleicher and Schuell, Dassel, Germany). After blocking with TTBS (10 mM Tris-HCl pH 7.4, 100 mM NaCl, 0.1% Tween 20) containing 5% low fat dry milk powder (TTBS-5% MP), the dot and Western blotted filters were incubated with respective antibodies in TTBS-1% MP for 60 min. Following three washes in TTBS, the filters were incubated with appropriate HRP-conjugated second antibodies. All incubations were carried out at room temperature. After thorough washing, the filters were developed with ECL reagent and exposed to Kodak X-Omat film. When required, the luminograms were quantitated using a laser scanning densitometer (Molecular Dynamics, Sunnyvale, CA).

2.4. Cholera toxin binding to membrane components in gradient fractions

CT binding to membrane-associated ganglioside GM1 in gradient fractions was evaluated by incubating the dot-blotted nitrocellulose filters, after blocking in 5% MP-TTBS, with HRP-conjugated CT in 1% MP-TTBS at 1:20 000 dilution. After washing in TTBS, filters were developed with ECL reagent.

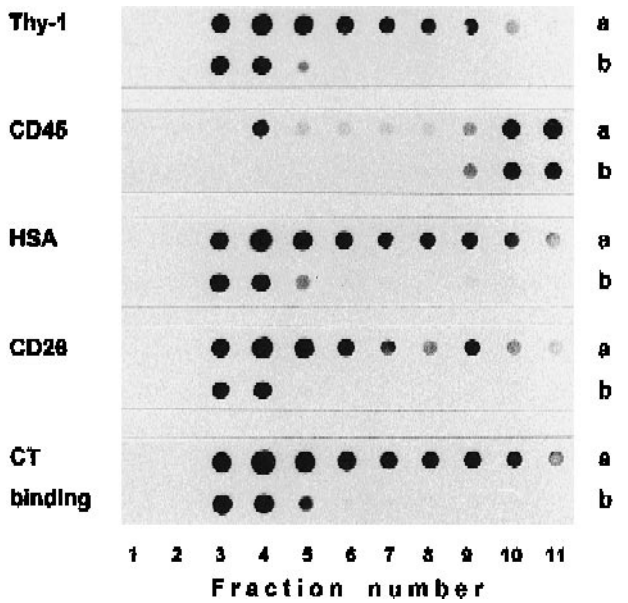

Fig. 1. Distribution of various cell surface markers in density gradient fractions of OTG lysates of lymphocytes. Two hundred million (a) or 50 million (b) P1798 cells were lysed in 0.75 ml of TKM buffer containing 60 mM OTG at 4°C and subjected to gradient centrifugation as described in Section 2: Materials and methods. One-ml fractions were collected from the top and 10 ml of each fraction was tested for the presence of Thy-1, HSA, CD26 or cholera toxin binding ganglioside by dot immunoassay as detailed in Section 2. Representative results from two independent experiments are shown.

3. Results

3.1. Lymphocyte GPI domains are resistant to dissociation by OTG

Most of the procedures used to isolate GPI-rich membrane domains as floating fractions in equilibrium density gradients selectively employ TX-100 or closely related detergents, such as Nonidet P-40 for cell lysis [10–12,21]. Use of octylglucoside (OTG) has been shown to disrupt these floating complexes from MDCK cells rendering most of the GPI-anchored placental alkaline phosphatase (PLAP) stably expressed in these cells non-floatable in sucrose gradients [11]. However, when a 60-mM OTG lysate of murine T-lymphoma cell line P1798 was subjected to equilibrium gradient centrifugation, the GPI-anchored cell surface glycoproteins Thy-1 and HSA, and ganglioside GM1 floated to the light density fractions at the 5–36% interface (fractions 3–5) (Fig. 1a), while most of the transmembrane glycoprotein CD45 failed to float and remained in the sample loading zone (fractions 10–11). Interestingly, CD26, a type II transmembrane protein, was also found in GPI-rich fractions. In order to rule out the possibility that the ratio of OTG to cells used in these experiments was suboptimal for complete extraction, the same amount of lysis buffer was used on only one fourth of the cells. While the floatation of Thy-1 was restricted to a narrow region, the small amount of floating CD45 was dissociated and remained in the sample loading zone (Fig. 1b).

3.2. Lymphocyte Thy-1 and endothelial cell CD59 display different floatation properties in sucrose gradients

In order to investigate the properties of lymphocyte GPI domains further, we compared a GPI-anchored and a transmembrane glycoprotein from P1798 cells (Fig. 2) and ECV304 endothelial cells (Fig. 3) under different conditions of detergent lysis.

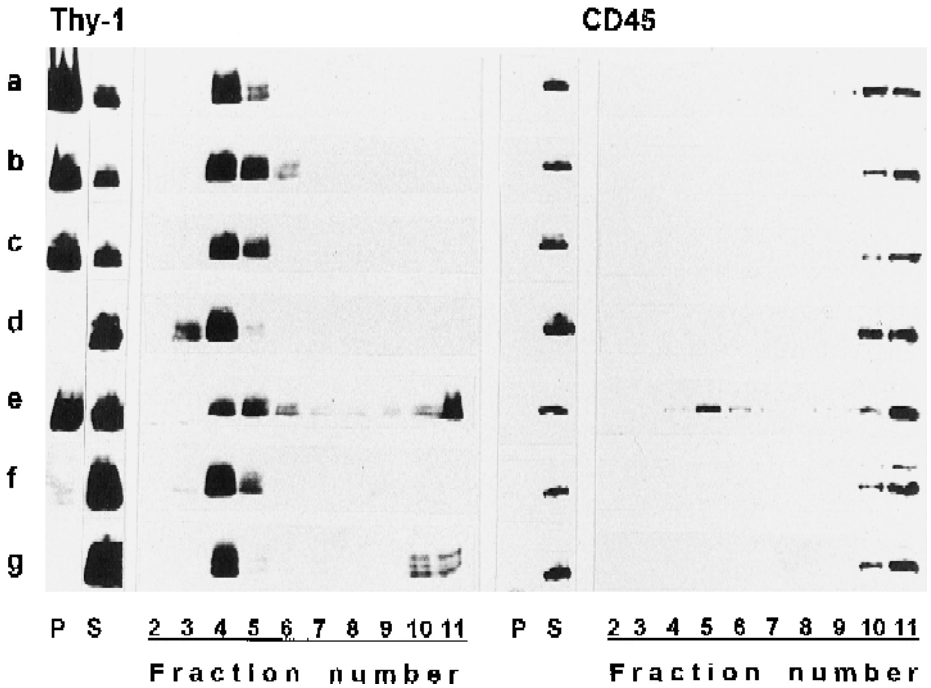

Fig. 2. Extractability and floatability of a GPI-anchored and a transmembrane lymphocyte surface molecule. One hundred million P1798 cells were lysed in 0.750 ml of TKM buffer with: (a) 0.5% TX-100 at 4°C; (b) 1.0% TX-100 at 4°C; (c) 2.0% TX-100 at 4°C; (d) 1.0% TX-100 at 37°C; (e) 30 mM OTG at 4°C; (f) 60 mM OTG at 4°C; or (g) 120 mM OTG at 4°C for 20 min. The lysates were subjected to sucrose density gradient centrifugation as described in Section 2: Materials and methods. Ten μl of the postnuclear detergent extract (S, supernatant) or SDS solubilized nuclear pellet (P, pellet), and 20 ml of the density gradient fractions (2–11) were mixed with 6 × non-reducing sample buffer, electrophoresed in 12% SDS–PAGE for Thy-1 and 10% gel for CD45, blotted onto nitrocellulose filters and the proteins were detected as described in Section 2. Data shown are representative of at least three experiments.

Increasing the concentration of TX-100 from 0.5 to 2% did not increase the amount of Thy-1 released in lymphocyte postnuclear supernatants (Fig. 2a–c; S vs. P), and endothelial cell CD59 behaved like Thy-1 under these extraction conditions (Fig. 3a–c; S vs. P). Interestingly, while almost all of lymphocyte Thy-1 was released by 1% TX-100 at 37°C, the amount of CD59 released from ECV304 cells under the same conditions was only 50% (Figs. 2 and 3d). In comparison, increasing the OTG concentration from 30 to 60 mM already released all of Thy-1 and CD59 into the postnuclear supernatants (Figs. 2 and 3e–g). However, striking differences were observed in the floatation properties of the released GPI-anchored proteins from lymphocytes and endothelial cells (Figs. 2 and 3). Fraction 1 (not shown) did not contain any detectable cell surface marker. All the TX-100 released Thy-1 floated to the top of the gradient (interface between 5 and 36% sucrose), irrespective of detergent concentration and temperature during lysis. CD59 released by 0.5% TX-100 at 4°C was completely floatable (Fig. 3a), but increasing the TX-100 concentration resulted in a decrease in the amount of floating CD59 (Fig. 3b–c) and this effect even was more pronounced when the cell lysis was carried out at 37°C (Fig. 3d). About 50% of Thy-1 and 70% of CD59 floated up when the cells were lysed in 30 mM OTG, and increasing the OTG concentration caused CD59 to dissociate from the floating fraction while the amount of floatable of Thy-1 was actually increased.

In contrast to the GPI-anchored proteins, the transmembrane CD45 (lymphocyte) and major histocompatibility class-I (endothelial cell) proteins were completely extracted by both TX-100 and OTG (Figs. 2 and 3), except MHC-I in low detergent concentrations of 0.5% TX-100 or 30 mM OTG. Most of the extracted CD45 and MHC-I remained at the bottom of the gradient, in agreement with earlier observations [11]. However, it should be noted that approximately 10% of the transmembrane CD45 and MHC-I molecules floated up when cells were lysed with 30 mM OTG (Figs. 2 and 3e), as when 200×10^6 cells were lysed in the same volume of 60 mM OTG (Fig.

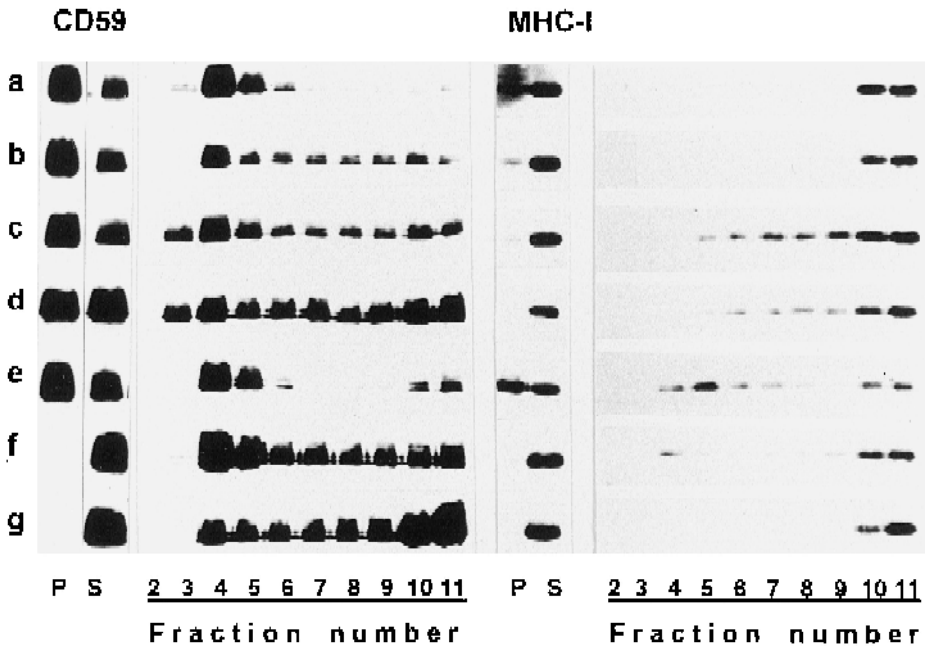

Fig. 3. Extractability and floatability of a GPI-anchored and a transmembrane endothelial cell surface molecule. ECV304 cells grown in 10-cm Petri dishes were lysed in 0.75 ml (per plate) of TKM containing: (a) 0.5% TX-100 at 4°C; (b) 1.0% TX-100 at 4°C; (c) 2.0% TX-100 at 4°C; (d) 1.0% TX-100 at 37°C; (e) 30 mM OTG at 4°C; (f) 60 mM OTG at 4°C; or (g) 120 mM OTG at 4°C for 20 min. The lysates were subjected to sucrose density gradient centrifugation as described in Section 2: Materials and methods. Ten μl of the post nuclear detergent extract (S, supernatant) or SDS solubilized nuclear pellet (P, pellet), and 20 ml of the density gradient fractions (2–11) were mixed with 6 × non-reducing sample buffer, electrophoresed in 15% SDS–PAGE for CD59 and 10% gel for MHC-I, blotted onto nitrocellulose filters and the proteins were detected as described in Section 2.

1). It has been reported earlier that another transmembrane protein CD44, in TX-100 lysates of fibroblasts, floated up in sucrose density gradients [35]. In Fig. 4, we analysed the distribution of CD44 in various gradient fractions of ECV304 cells, and interestingly over 50% of CD44 was found to be associated with the floating fractions even after extraction with 60 mM OTG (Fig. 4f).

3.3. The GM1 ganglioside from lymphocytes and endothelial cells is essentially floatable

Since sphingolipids are believed to contribute to the formation of GPI domains in mammalian cell plasma membrane [11,17,36], we evaluated the floatability of cholera toxin binding ganglioside GM1 from lymphocytes and endothelial cells. Fig. 5 clearly shows that the bulk of the cholera toxin binding activity in P1798 lymphoma cells floated up after both TX-100 and OTG extraction. Identical results were observed with ECV304, except that a substantially higher amount of CT binding activity was recovered in the bottom fractions of OTG lysates.

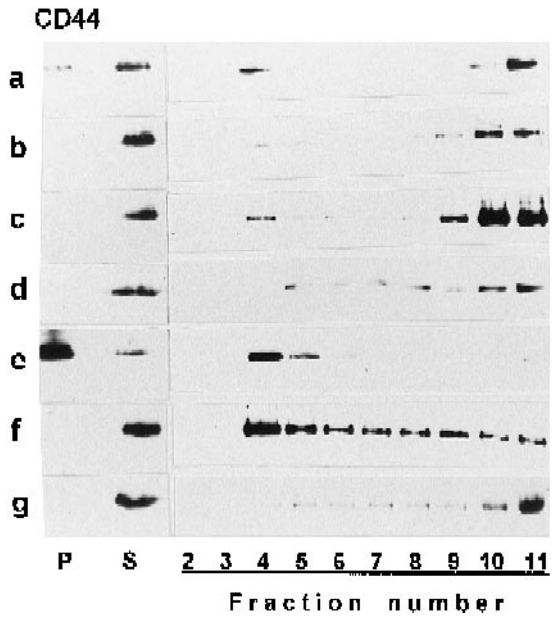

Fig. 4. Extraction and floatation properties of CD44 ECV304 cells. The samples in Fig. 3 are analysed for the distribution of CD44 in 10% gels under non-reducing conditions. Rows a–g as in Fig. 3.

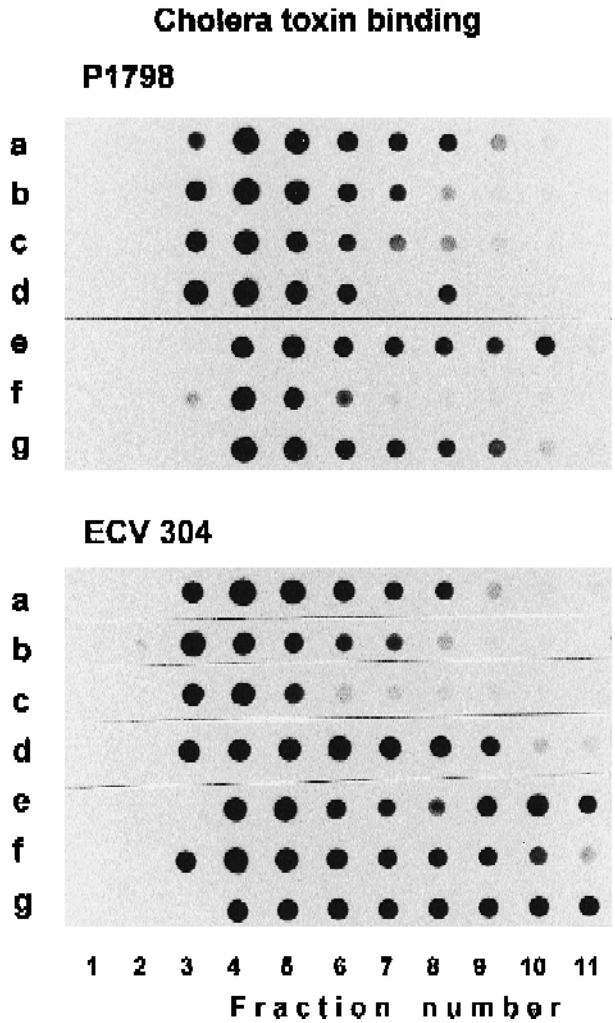

Fig. 5. Floatation properties of cholera toxin binding activity in the density gradient fractions of P1798 and ECV304 under different conditions of detergent extraction. Ten μl of the gradient fractions used in Fig. 2Fig. 3 were dot-blotted onto nitrocellulose filters and probed with HRP-conjugated cholera toxin as described in Section 2.

3.4. Intracellular acylated molecules display variable degrees of association with GPI domains

Next we examined, under similar extraction conditions, the floatability of intracellular acylated molecules known to associate with the inner leaflet of the plasma membrane GPI domains. The Src family protein tyrosine kinase Lck from P1798 lymphoma cells was almost completely extracted under all ex-

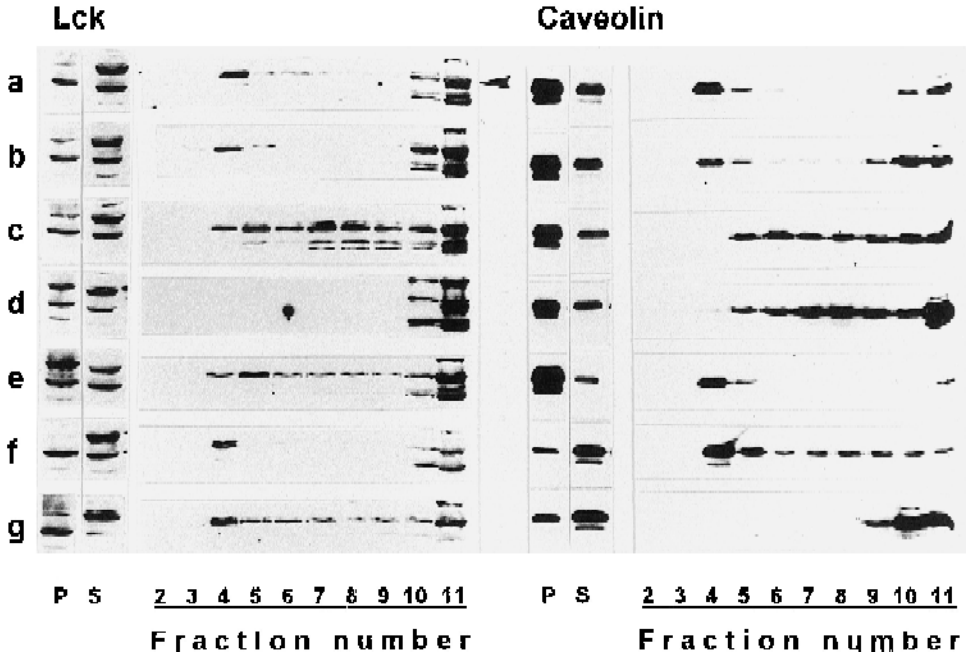

Fig. 6. Extractability of p56^{lck} and caveolin under different conditions of detergent lysis, and their floatability in sucrose density gradients. Samples from the sucrose density gradient shown in Fig. 2Fig. 3 were electrophoresed in 10 or 15% SDS–PAGE under reducing conditions, blotted onto nitrocellulose filters and probed for p56^{lck} and caveolin, respectively. For details see Fig. 2Fig. 3. The band corresponding to the 56-kDa Lck protein is marked with an arrowhead.

traction conditions at 4°C, but only partially with 30 mM OTG (Fig. 6e; 56-kDa band) and 25–50% of the extracted p56^{lck} always floated to top fractions under all extraction conditions at 4°C (Fig. 6). In comparison, caveolin in ECV304 cells behaved almost identically to CD59 in its gradient distribution (Figs. 3 and 6), but unlike Lck, caveolin in the floating fractions was resistant to dissociation by TX-100 extraction at 37°C (Fig. 6d). However, virtually all caveolin failed to float in 120 mM OTG, while the floating fraction of Lck remained unaffected.

4. Discussion

GPI domains and caveolae have been recently recognized as lipid–protein scaffolds assembling signalling molecules in the mammalian cell plasma membrane [29,37,38]. However, the precise nature of these membrane specializations, and their role in the regulation of cellular responses to extracellular stimuli remain poorly defined [27]. Characterization of GPI domain structures principally relies on their biophysical properties [10–12,33]. Caveolae also share these properties [19,21], but morphological studies have clearly distinguished them from GPI domains [23,24]. Several signal-transducing membrane components of the outer and inner leaflets are associated with GPI-anchored proteins and caveolin [21,39–42]. In an attempt to develop optimal conditions for studying the physical and functional properties of GPI anchored proteins and their relationship to other membrane-associated extracellular and intracellular components, we compared GPI domains from non-polarized lymphocytes and polarized endothelial cells under various conditions of detergent lysis that were reported to differentially affect the integrity of GPI domains [11,28].

Both lymphocyte Thy-1 and endothelial CD59 were only partially released into post nuclear supernatants by TX-100 at 4°C, over a broad range of detergent concentrations, as demonstrated for several other GPI-anchored proteins from a variety of cell types [2–8]. However, Thy-1 was completely extracted by TX-100 at 37°C like PLAP expressed on MDCK cells [11], while endothelial CD59 was not. Interest-

ingly, Thy-1 floated as light density complexes under all extraction conditions including 120 mM OTG, while CD59-containing complexes of endothelial cells were dissociated from buoyant membrane lipids at higher concentrations of TX-100 or OTG, as PLAP in MDCK cells [11]. Glycosphingolipid GM1 has been demonstrated to be part of GPI domains and caveolae [11,14,15,33]. GM1 from lymphocytes and endothelial cells showed nearly identical floatation behaviour in sucrose gradients. However, a significant amount of GM1 in the non-floating fractions of OTG lysates of ECV304 cells may probably arise from their tight association with the dissociated CD59. These results indicate that a given GPI-anchored protein remains floatable to varying extents, probably because detergents differentially affect its interactions with glycosphingolipids. For example, OTG almost completely extracted PLAP from the light density GPI-rich apical membrane complexes of MDCK cells, but not the sphingolipids [28]. Therefore, it seems likely that the interactions between the acyl chains of GPI-anchors and GSLs within the membrane, as well as the extracellular interactions of GPI-anchored proteins and GSLs could vary significantly resulting in heterogeneous extraction and floatation behaviour.

Several lipid-modified intracellular signalling proteins such as Src-family protein tyrosine kinases, heterotrimeric G-proteins and Ras have been shown to associate with detergent-insoluble GPI domains and caveolae [21,31,39,41,42]. Whether the inner leaflet membrane corresponding to the GPI/GSL and caveolin-rich plasma membrane domains is unique in lipid composition and could provide a different mode of interaction for the acylated signalling molecules is not yet known [27]. The association of caveolin with the floating fractions in endothelial cells seems to be less strong than that of Lck. In our experiments, a significant (25–50%) amount of $p56^{lck}$ was always associated with the light density floating material, and was separated from it only by TX-100 at 37°C. Caveolin in the floating fractions was relatively resistant to disruption by TX-100 extraction at 37°C, but was completely dissociated by OTG. These floatation differences could be due to the way these molecules interact with the plasma membrane. The interaction of Src-family PTKs with GPI domains is dependent on N-terminal myristoylation and palmitoylation [43], as well as stretches of basic amino acids at the amino terminal ends that strengthen their membrane association through interaction with acidic phospholipids of the inner leaflet [44]. Caveolin has an intramembranous segment, palmitoylated on multiple cysteine residues, and forms homo-oligomers [45,46] that might influence its association with the hydrophobic core of the membrane bilayer [47]. Expression of VIP21-caveolin in lymphocytes results in the formation of morphologically defined caveolae [48], but the question as to whether expression of caveolin modulates the interactions of GPI anchors with GSLs, and the susceptibility of the GPI domains to detergent solubilization has not been addressed.

Transmembrane glycoproteins are usually excluded from the floating GPI/GSL and caveolin-rich membrane fractions [11,19,33]. However, certain transmembrane proteins associate with GPI domains, notably CD26 in intestinal brush border membranes and lymphocytes [8,33], CD36 in endothelial cell [19] and CD44 in fibroblasts [35]. It should be noted that lymphocyte CD26 was not at all dissociated from the floating fractions even after OTG extraction (Fig. 1) and endothelial CD44 only partially (Fig. 4), while CD45 and MHC-I molecules from these two cell types were completely dissociated (Figs. 2 and 3). CD26 is a type II transmembrane protein, while CD36 and CD44 contain acyl modifications at N- and C-terminal ends, respectively [19,49]. Interestingly, CD45 was co-isolated with Thy-1 after chemical cross-linking [50], and we have also observed that a significant amount of CD45 is consistently present in floating membrane complexes when cell homogenate, rather than detergent lysate, is used (data not shown). In addition, GPI anchored molecules have been shown to interact functionally with transmembrane signal transducers, such as integrins [51] and receptor protein tyrosine kinases [52]. These observations suggest that transmembrane glycoproteins interact with GPI domains at their periphery, for instance with the carbohydrate moieties of GSLs or GPI-anchored proteins, and these interactions are disrupted to a variable extent by different detergents.

A number of studies have shown that plasma membrane GPI domains and caveolae can be isolated without using any detergent [29,30,53], and caveolae free of GPI domains can also be obtained [54]. These studies argue against the possibility that GPI domains could be detergent-induced artifacts, although the

co-isolation of GPI-anchored proteins and caveolae could arise from their tendency to co-aggregate following exposure to detergents [55]. Furthermore, the detergent-free isolation techniques have revealed additional molecular interactions, both on the outer and inner leaflets of GPI domains [30,54]. Therefore, it is likely that the detergent-insoluble fractions could represent only the core of the dynamic GSL-rich membrane domains, after dissociation of peripherally associated cell surface molecules. The core GPI domain arriving at the plasma membrane as GPI/GSL-rich raft may differ among the various cell types: the size, glycosylation and conformation of GPI-anchored proteins could vary greatly [1], as much as the nature and amount of extracellular carbohydrates in gangliosides and membrane cholesterol content [27]. It is conceivable that these factors profoundly influence: (1) transmembrane interactions between the acyl chains of GPI anchors and cell type-specific lipids; and (2) extracellular interactions of GPI-anchored proteins with GSLs in the GPI domain, and with transmembrane glycoproteins at the periphery of GPI domains. For example, a strong lipid–GPI acyl chain interaction would result in their floatation whereas, a strong extracellular interaction of a GPI-anchored protein with a transmembrane glycoprotein could lead either to inclusion of the latter into the rafts, or dissociation of the former from the rafts. Certainly, several GPI-anchored proteins in different cell types need to be analysed, in addition to analysing the GPI-anchored proteins of one cell type expressed in another.

Acknowledgements

This work was supported by the Swiss National Science Foundation (Grant 31-39 709.93) and Société Académique de Genève. S.I. is a recipient of a fellowship from The Sir Jules Thorn Charitable Trusts.

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