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Polarized apical distribution of glycosyl-phosphatidylinositol-anchored proteins in a renal epithelial cell line

(plasma membrane/protein sorting/membrane glycoproteins/phosphatidylinositol-specific phospholipase C/sulfosuccinimidobiotin)

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ABSTRACT **Polarized epithelial cell monolayers contain two distinct plasma membrane domains as delineated by the presence of tight junctions—i.e., an apical surface that faces the external environment and a basolateral surface that functions both in cell–cell contact and cell–substrate attachment. Central to the understanding of epithelial cell polarity is the question of how such cell-surface specializations are generated. A different class of membrane glycoproteins has recently emerged that may yield new insight into the mechanism underlying the biogenesis of this polarity. Members of this class contain a large extracellular protein domain linked to the membrane via glycosyl-phosphatidylinositol. Using a polarized renal epithelial cell line (Madin–Darby canine kidney), we identified endogenous glycosyl-phosphatidylinositol-anchored proteins through release by a phosphatidylinositol-specific phospholipase C. Six glycosyl-phosphatidylinositol-anchored proteins of 110, 85, 70, 55, 38, and 35 kDa were identified and appeared to be restricted to the apical surface. Our data are consistent with the notion that the glycosyl-phosphatidylinositol membrane anchor may contain the necessary information for "targeting" to the apical surface.**

Polarized epithelial cells act as a selective barrier in a variety of tissue types, allowing the directional transport of certain ions and macromolecules. This vectorial transport depends upon the existence of two distinct plasma membrane domains—i.e., an apical and a basolateral surface, as defined by the presence of tight junctions (1, 2). The apical membrane, which faces the external milieu, contains various morphological specializations, including microvilli and cilia (3, 4). In contrast, the basolateral membrane, which faces the internal milieu, has receptors for components of the basal lamina and interacts with neighboring cells via cell adhesion molecules and a variety of junctional elements (3, 4).

Central to the understanding of epithelial polarity is the question of how proteins are targeted and retained by specific plasma membrane domains. The observation that viral envelope glycoproteins are selectively targeted to either the apical or basolateral cell surface has been exploited to partially unravel the underlying mechanism (3, 5–10). Recombinant DNA technology has been utilized to localize the "sorting" signal to a specific glycoprotein domain—i.e., extracellular, transmembrane, or cytoplasmic. Although conflicting results exist, the extracellular domain appears to contain the information necessary for correct sorting (11–13). A similar conclusion can be drawn from the study of secretion. Certain secretory proteins, which possess only an extracellular domain, are secreted in a polarized fashion (14–16).

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The study of a different class of membrane glycoproteins may yield further insight into the mechanism of protein sorting. Members of this class contain a large extracellular protein domain linked to the membrane via glycosyl-phosphatidylinositol (glycosyl-PI) (17–20). This linkage involves the covalent attachment of the C-terminal amino acid (via ethanolamine) to an oligosaccharide chain, which in turn is linked to the inositol ring of phosphatidylinositol (PI). Although the basic structural features of this glycolipid have been elucidated (17–20), the precise function of this mode of protein anchoring is unknown. Therefore, we explored the possibility that putative sorting signals might reside in the polypeptide backbone and/or the glycolipid membrane anchor.

In this paper, we report the preferred apical distribution of glycosyl-PI-anchored proteins in the well-characterized polarized renal epithelial cell line [Madin–Darby canine kidney (MDCK)] (3–9, 11–16). These results suggest a possible role for glycosyl-PI in "targeting" these glycoproteins to the apical membrane.

MATERIALS AND METHODS

Reagents. Sulfosuccinimidobiotin (sulfo-NHS-biotin) biotinylating agent was purchased from Pierce. PI-specific phospholipase C (PI-PLC), purified from *Bacillus thuringiensis*, was the gift of Martin Low (Columbia University College of Physicians and Surgeons, New York) and contained no detectable protease activity (21, 22). Streptavidin and molecular mass markers were obtained from Bethesda Research Laboratories.

Cells and Cell Culture. Human erythrocytes were prepared from freshly collected citrated blood (23). PC-12 cells were maintained in 100-mm plastic Petri dishes with Dulbecco's modified Eagle's (DME) medium supplemented with 10% (vol/vol) fetal calf serum and 5% (vol/vol) horse serum (GIBCO) (24).

MDCK cells, type II, were seeded at high density in 24.5-mm polycarbonate filter chambers (Transwell tissue culture treated inserts, 0.45 μm; Costar, Cambridge, MA) and maintained in DME medium containing 10% horse serum and antibiotics (GIBCO) for 5–7 days to allow the development of a tight monolayer (25). Approximately 12–16 hr before processing, MDCK cells were serum-starved in DME medium containing 0.2% bovine serum albumin. Monolayer "permeability" was assessed by monitoring the diffusion of [^{3}H]inulin (New England Nuclear, Chadds Ford, PA) from the apical to the basolateral compartment (26). Filter-grown

Abbreviations: PI, phosphatidylinositol; glycosyl-PI, glycosyl-phosphatidylinositol; MDCK, Madin–Darby canine kidney; PI-PLC, phosphatidylinositol-specific phospholipase C; sulfo-NHS-biotin, sulfosuccinimidobiotin.

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MDCK cell monolayers with a permeability of >1% (after 2 hr at 37°C) were discarded.

Cell-Surface Labeling. After repeated washings (five times) with ice-cold phosphate-buffered saline (PBS) containing 0.1 mM $CaCl_2$ and 1 mM $MgCl_2$ (PBS-C/M), cells were treated with sulfo-NHS-biotin in PBS-C/M (0.5 mg/ml) at 4°C (27, 28). After 30 min, cells were washed once with ice-cold serum-free DME medium and four times with PBS-C/M.

For selective labeling of the apical or the basolateral surface, sulfo-NHS-biotin was added either to the apical or the basolateral compartment of the filter chamber (29, 30). The compartment not receiving sulfo-NHS-biotin was filled with an equivalent volume of PBS-C/M. Three filter chambers (7.2×10^6 cells) were used per experimental condition.

Cell Lysis and PI-PLC Treatment. PI-PLC treatment was similar to a method described previously (31–34) with certain critical modifications. Cells ($\approx 10^7$ human erythrocytes, 5×10^6 PC-12 cells, or 7.2×10^6 MDCK cells) were extracted with 1 ml of Tris-buffered saline (TBS; 10 mM Tris, pH 7.4/0.15 M NaCl/1 mM EDTA) containing 1% (vol/vol) Triton X-114 for 1 hr at 4°C with mild agitation. Cell extracts were clarified by centrifugation (14,000 × *g* for 10 min) at 4°C. Resulting supernatants were partitioned into detergent and aqueous phases by temperature-induced phase separation (34). Aqueous phases were discarded, and detergent phases were reextracted three times with 10 vol of TBS containing 0.06% Triton X-114. In between additional phase separations, diluted detergent phases were clarified by centrifugation (14,000 × *g* for 5 min) at 4°C.

Reextracted detergent phases (100 μl), enriched with membrane (hydrophobic) forms of glycosyl-PI-anchored proteins, were diluted with 100 mM Tris, pH 7.4/50 mM NaCl/1 mM EDTA to a final volume of 0.5 ml. Diluted detergent phases, as such, were then incubated in the absence or presence of PI-PLC (6 units/ml) with continuous mixing in an Eppendorf mixer (5432) for 1 hr at 37°C. Immediately after PI-PLC treatment, samples were diluted to a final volume of 1 ml with TBS containing 2% Triton X-114 and subjected to temperature-induced phase separation. Detergent phases were discarded, and aqueous phases [containing soluble (hydrophilic) forms of glycosyl-PI-anchored proteins resulting from PI-PLC treatment] were collected. Aqueous phases were reextracted three times by the addition of 100 μl of 10% Triton X-114 in TBS (without EDTA), followed by temperature-induced phase separation.

Resulting aqueous phases were quantitatively precipitated by using sodium deoxycholate at 125 μg/ml and 6% (wt/vol) trichloroacetic acid (35). After solubilization in Laemmli sample buffer, excess acid was neutralized with NH_4OH vapors. It should be noted that all solutions used for cell extraction, phase separation, and PI-PLC treatment were ice-cold and contained each of the following protease inhibitors at 10 μg/ml: leupeptin, pepstatin A, and antipain.

Detection of Biotinylated Proteins. Samples were subjected to $NaDodSO_4$/PAGE as described by Laemmli (36) and transferred to nitrocellulose (37). After incubation of nitrocellulose sheets with ^{125}I-labeled streptavidin [$1–2 \times 10^6$ cpm/ml in PBS containing 0.5% (vol/vol) Tween 20, 1 M D-glucose, 10% (vol/vol) glycerol, and 0.3% bovine serum albumin] under conditions that reduce nonspecific binding (37), biotinylated proteins were visualized by autoradiography on Kodak XAR-5 film (4–24 hr at −80°C with an intensifying screen).

Scanning of autoradiographs was performed by using a GS-300 transmittance/reflectance scanning densitometer (Hoefer, San Francisco). The most prominent band in the lane corresponding to the PI-PLC-treated sample was adjusted to 90% absorbance and compared with untreated controls. Scans corresponding to PI-PLC-treated samples and untreated controls were then automatically subtracted by using the GS-365 Data System/IBM PC version.

Iodinated streptavidin was prepared by using $Na^{125}I$ (New England Nuclear) and chloramine T, yielding a specific activity of 5 μCi/μg (1 μCi = 37 kBq). Molecular mass markers are as indicated and are expressed in kDa: myosin heavy chain, 200; phosphorylase *b*, 97.4; bovine serum albumin, 68; ovalbumin, 43; α-chymotrypsinogen, 25; β-lactoglobulin, 18.4; and lysozyme, 14.3.

RESULTS

Detection of Glycosyl-PI-Anchored Proteins. We have chosen to identify glycosyl-PI-anchored proteins by exploiting three conserved structural features, namely: (*i*) their presence solely on the exoplasmic leaflet of the lipid bilayer; (*ii*) the susceptibility of the glycosyl-PI membrane anchor to cleavage by PI-PLC; and (*iii*) the PI-PLC-induced transition from a hydrophobic to a hydrophilic state because of loss of a putative detergent binding domain, i.e., diacylglycerol.

We used an experimental approach that combines cell-surface labeling, Triton X-114 phase separation, and PI-PLC treatment. Briefly, cells were surface-labeled with a membrane-impermeant marker (sulfo-NHS-biotin) that reacts specifically with free amino groups. After extraction and phase separation with the detergent Triton X-114, detergent phases enriched in membrane (hydrophobic) forms of glycosyl-PI-anchored proteins were treated with PI-PLC. The generation of soluble (hydrophilic) forms of glycosyl-PI-anchored proteins by PI-PLC was monitored by their subsequent partitioning into the aqueous phase during a final phase separation. After $NaDodSO_4$/PAGE and transfer to nitrocellulose, the biotinylated proteins were visualized with ^{125}I-labeled streptavidin. Autoradiographs were scanned, and the lanes corresponding to PI-PLC-treated samples and untreated controls were automatically subtracted to allow a more quantitative view of proteins specifically released by PI-PLC. A similar scheme, except for the use of lactoperoxidase-catalyzed iodination as a labeling system, has been used (34) to identify glycosyl-PI-anchored proteins, including Thy-1, in lymphoma cell lines.

Cell types containing known glycosyl-PI-anchored proteins were used as controls to evaluate the sensitivity of the detection system. Human erythrocytes were used since they contain known glycosyl-PI-anchored proteins in a form partially resistant to cleavage by PI-PLC. Only 10–15% of decay-accelerating factor and acetylcholinesterase (both 70 kDa) can be released upon treatment of human erythrocytes with PI-PLC (23, 38, 39). Such resistance is apparently due to selective acylation of the inositol ring (40). Human erythrocytes were surface-labeled in suspension, extracted with detergent, and treated with PI-PLC. By comparison of PI-PLC-treated samples and untreated controls, a major band specifically released by PI-PLC corresponding to the known molecular mass of acetylcholinesterase or decay-accelerating factor was observed (Fig. 1). Thus, the assay system appears sensitive enough to detect even minor amounts of released PI-PLC-sensitive glycosyl-PI-anchored proteins.

PC-12 cells were used as an additional positive control because they contain Thy-1, a known glycosyl-PI-anchored protein of 24 kDa (41–43). A number of biotinylated proteins (60, 40, and 24 kDa) were specifically released by PI-PLC. As expected, the most prominent band corresponded to the known molecular mass of Thy-1 (24 kDa) (Fig. 2).

In untreated extracts, a number of biotin-labeled proteins were spontaneously released (independently of PI-PLC) from the detergent to the aqueous phase. As observed previously (34), a background level caused by the partitioning of membrane proteins into the aqueous phase is unavoidable because this phase contains ≈0.7 mM Triton X-114, which is 3 times

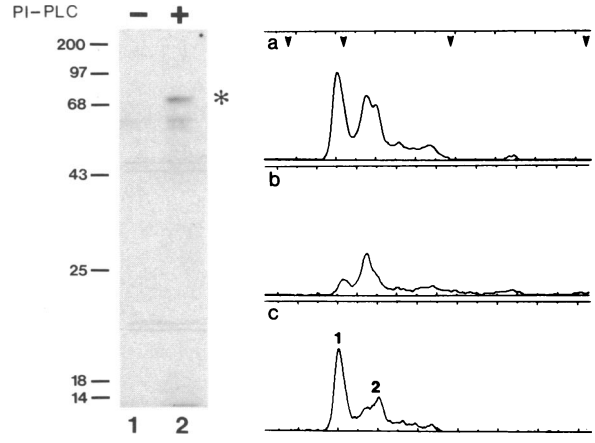

FIG. 1. Identification of glycosyl-PI-anchored proteins in human erythrocytes. (*Left*) A major glycosyl-PI-anchored protein corresponding to the known molecular mass of decay-accelerating factor or acetylcholinesterase (70 kDa) is indicated by an asterisk. Human erythrocytes (10^7 cells) were surface-labeled in suspension with sulfo-NHS-biotin (0.5 mg/ml in PBS-C/M; 30 min at 4°C). After detergent extraction and phase separation, samples were treated in the absence (lane −) or presence (lane +) of PI-PLC (6 units/ml). After $NaDodSO_4$/PAGE (10%) and transfer to nitrocellulose, biotinylated proteins were visualized with ^{125}I-labeled streptavidin and autoradiography. Molecular masses are indicated in kDa. (*Right*) Densitometric analysis of *Left*. The autoradiograph was scanned, and lanes corresponding to PI-PLC-treated samples and untreated controls were automatically subtracted by the computer. (*a*) Lane 2. (*b*) Lane 1. (*c*) Subtraction of *b* from *a*. A major peak corresponding to the known molecular mass of decay-accelerating factor or acetylcholinesterase was identified in *c* (peak 1). Arrowheads in *a* indicate the position of size markers—i.e., 97, 68, 43, and 25 kDa from left to right.

above the critical micellar concentration of this detergent and is sufficient to solubilize some plasma membrane proteins of intermediate hydrophobicity (see ref. 32 for a discussion of this point). Alternatively, spontaneous release might be the result of proteolytic or endogenous glycosyl-PI-PLC activity. Furthermore, the efficiency of Triton X-114 phase separation is somewhat variable, depending upon the precondensation temperature, the ionic strength, and the temperature during phase separation (44). Several measures were successfully used to minimize this spontaneous release and to maximize PI-PLC-specific release: (*i*) repeated sample clarification (centrifugation at 14,000 × *g* for 5 min at 4°C); (*ii*) serum starvation (12–16 hr in DME medium containing 0.2% bovine serum albumin), thereby preventing loss of glycosyl-PI-anchored proteins via hormone-mediated release (45) or via serum PI-specific phospholipase D (46); and (*iii*) inclusion of multiple protease inhibitors during all experimental manipulations.

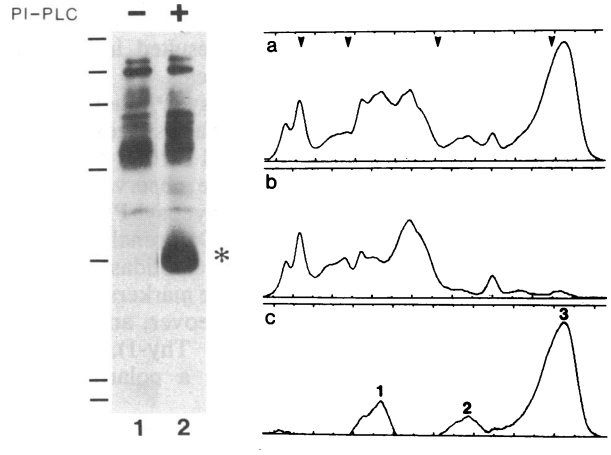

FIG. 2. Identification of glycosyl-PI-anchored proteins in PC-12 cells. (*Left*) A major glycosyl-PI-anchored protein corresponding to the known molecular mass of Thy-1 (24 kDa) is indicated by an asterisk. PC-12 cells (5×10^6) were surface-labeled with sulfo-NHS-biotin. Samples were treated in the absence (lane −) or present (lane +) of PI-PLC (6 units/ml). Size markers are as indicated in Fig. 1 *Left*. (*Right*) Densitometric analysis of *Left*. The autoradiograph was scanned, and lanes corresponding to PI-PLC-treated samples and untreated controls were automatically subtracted. (*a*) Lane 2. (*b*) Lane 1. (*c*) Subtraction of *b* from *a*. A major peak corresponding to the known molecular mass of Thy-1 was identified in *c* (peak 3). Other proteins specifically released by PI-PLC were of 60 and 40 kDa (peaks 1 and 2 in *c*). Arrowheads in *a* indicate the position of size standards as described in the legend of Fig. 1 *Right*.

Preferred Apical Distribution of Glycosyl-PI-Anchored Proteins in MDCK Cells. To assess the polarized distribution of glycosyl-PI-anchored proteins, MDCK cells were grown to confluence (5–7 days) in polycarbonate filter chambers, which allow separate access to the apical and the basolateral surfaces. MDCK cells grown on such permeable supports were then selectively labeled either on the apical or the basolateral surface by using sulfo-NHS-biotin. Filters were then excised, and the proteins were extracted with Triton X-114 and subjected to temperature-induced phase separation. Aqueous and detergent phases, obtained from apically or basolaterally labeled monolayers, were then acetone-precipitated (5 vol) and subjected to $NaDodSO_4$/PAGE and ^{125}I-labeled streptavidin blotting. Strikingly different patterns of biotinylated "peripheral" and "integral" membrane proteins were visualized when apically labeled monolayers were compared with their basolaterally labeled counterparts, confirming that such labeling is confined to a specific cell surface (Fig. 3). Recent experiments with semithin frozen sections have shown that labeling is indeed confined to the apical or basolateral surface and is uniform up to the level of the tight junction (M.S., M.P.L., L.G., A. LeBivic, and E.R.-B., unpublished data.)

After selective labeling of the apical or the basolateral cell surface, filter-grown monolayers were detergent-extracted and subjected to PI-PLC treatment. Comparison of PI-PLC-treated samples and untreated controls of apically labeled monolayers revealed six glycosyl-PI-anchored pro-

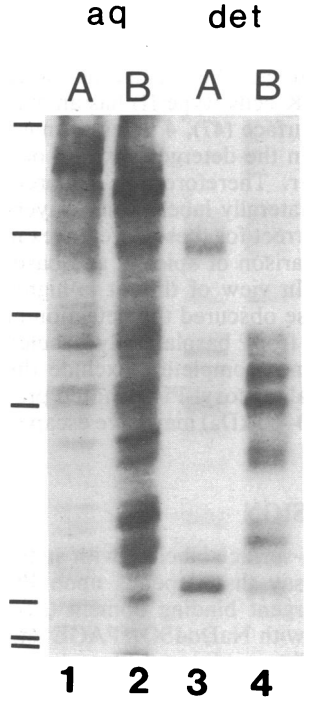

FIG. 3. Selective labeling of the apical or basolateral surface of the MDCK cell monolayers. Filter-grown (polycarbonate) MDCK cell monolayers (5–7 days confluent) were washed four times with PBS-C/M. Sulfo-NHS-biotin (0.5 mg/ml in PBS-C/M; 30 min at 4°C) was then added to the apical (lanes A) or basolateral (lanes B) compartment of the filter chamber. After extraction and phase separation with Triton X-114, aqueous (aq) (lanes 1 and 2) and detergent (det) phases (lanes 3 and 4) were acetone-precipitated (5 vol at −20°C for 30 min) and subjected to $NaDodSO_4$/7.5% PAGE. After transfer to nitrocellulose, biotinylated proteins were visualized with ^{125}I-labeled streptavidin. Note that protein profiles for apically (lanes A) and basolaterally (lanes B) labeled monolayers appear distinct. Molecular mass standards are as indicated in Fig. 1 *Left*.

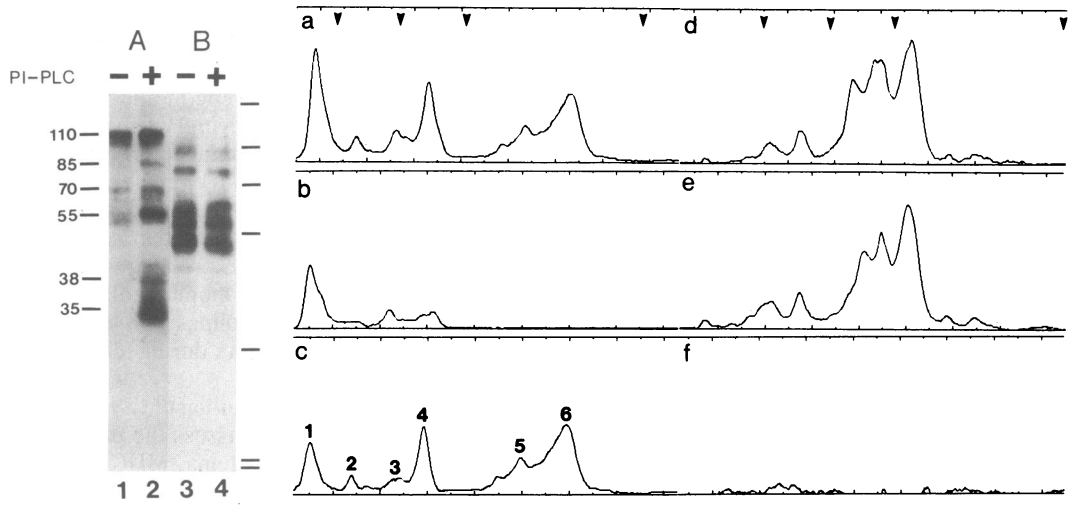

FIG. 4. Identification of glycosyl-PI-anchored proteins in "tight" MDCK monolayers. (*Left*) MDCK monolayers, 5–7 days confluent, were apically (lanes A) or basolaterally (lanes B) biotinylated. Detergent phases (Fig. 3, lanes 3 and 4), enriched in membrane forms of glycosyl-PI-anchored proteins, were treated in the absence (lanes −) or presence (lanes +) of PI-PLC (6 units/ml). After $NaDodSO_4$/10% PAGE, biotinylated proteins were visualized as described in the legend of Fig. 3. Soluble (hydrophilic) forms of glycosyl-PI-anchored proteins are indicated by bars with their apparent molecular mass in kDa. Three filters (7.2×10^6 cells) were used per experimental condition. Size markers on the right are as described in the legend of Fig. 1 *Left*. (*Right*) Densitometric analysis of *Left*. The autoradiograph was scanned, and lanes corresponding to PI-PLC-treated samples and untreated controls were automatically subtracted. (*a*) Lane 2. (*b*) Lane 1. (*c*) Subtraction of *b* from *a*. (*d*) Lane 4. (*e*) Lane 3. (*f*) Subtraction of *e* from *d*. Computer subtraction of apically biotinylated samples (*c*) revealed six glycosyl-PI-anchored proteins. Peaks numbered 1–6 correspond to 110, 85, 70, 55, 38, and 35 kDa, respectively. No glycosyl-PI-anchored proteins were observed when computer subtraction of basolaterally biotinylated samples was performed (*f*). Arrowheads in *a* and *d* indicate the position of size markers—i.e., 97, 68, 43, and 25 kDa from left to right.

teins. Three major proteins (of 110, 55, and 35 kDa) and three minor proteins (of 85, 70, and 38 kDa) were identified. The release of these proteins was maximal with PI-PLC at 4–6 units/ml (Fig. 4 *Left*, lanes 1 and 2, and *Right c*). In contrast, comparison of PI-PLC-treated samples and untreated controls from basolaterally labeled monolayers did not result in the identification of any major PI-PLC releasable proteins (Fig. 4 *Left*, lanes 3 and 4, and *Right f*). Only trace amounts of the 35-kDa glycosyl-PI-anchored protein species were detectable on overexposure of the autoradiograph. Repeated attempts at generating additional PI-PLC-specific release with up to 10 units of PI-PLC per ml were unsuccessful, suggesting that glycosyl-PI-anchored proteins (PI-PLC sensitive) are present in an apically polarized distribution.

It should be noted that the apparently greater spontaneous protein release from basolaterally labeled monolayers can be attributed to the larger surface area of this membrane. Since the basolateral surface of MDCK cells (type II) has an area 4 times larger than the apical surface (47), 4 times as much biotinylated protein is present in the detergent extract of a basolaterally labeled monolayer. Therefore, the apparent spontaneous release from basolaterally labeled monolayers should be reduced 4 times to correct for these differences in surface area for accurate comparison of apically and basolaterally labeled counterparts. In view of this, it is highly unlikely that spontaneous release obscured the detection of glycosyl-PI-anchored proteins from basolaterally labeled monolayers. However, we cannot completely exclude the possibility that minor basolateral glycosyl-PI-anchored proteins (primarily in the range of 40–60 kDa) may have escaped detection.

DISCUSSION

In this paper, we have used cell-surface labeling with sulfo-NHS-biotin and a sensitive assay that depends upon PI-PLC-specific release of a detergent binding domain (i.e., diacylglycerol), in combination with $NaDodSO_4$/PAGE and subtractive densitometry, to detect glycosyl-PI-anchored proteins in whole-cell lysates. Although this experimental approach does not rely upon the use of a specific antibody or enzymatic assay to detect a specific candidate protein, it is the most sensitive technique available to survey the surface proteins anchored in this fashion in a given cell type (34).

To assess the polarized distribution of glycosyl-PI-anchored proteins, "tight" MDCK monolayers were selectively labeled either on the apical or the basolateral surface. Six glycosyl-PI-anchored proteins of 110, 85, 70, 55, 38, and 35 kDa were identified from cells labeled at the apical surface. Strikingly, no glycosyl-PI-anchored proteins were detected on cells labeled at the basal surface, in spite of its 4 times larger surface area and protein mass, under conditions that resulted in facile and reproducible detection of the apical proteins.

The observation that several glycosyl-PI-anchored proteins are restricted to the apical cell surface suggests that this modification may function as an apical sorting signal. This hypothesis is further supported by the observation that a variety of ecto-enzymes that contain glycosyl-PI membrane anchors [alkaline phosphatase (58–70 kDa), renal dipeptidase (130 kDa), trehalase (96 kDa), and 5′ nucleotidase (73 kDa)], have been described as apical membrane markers in different epithelial cell types (3, 4, 48, 49). Moreover, an exogenous glycosyl-PI-anchored protein (human Thy-1), when expressed in transgenic mice, assumed a polarized apical distribution in renal epithelia (50).

Alternatively, glycosyl-PI-anchored proteins might be delivered equally to both epithelial cell surfaces but then undergo a selective modification, degradation, or release at the basolateral surface, rendering them undetectable by the criterion of exogenous PI-PLC-induced release. Selective removal of glycosyl-PI-anchored proteins by an endogenous, hormonally activated glycosyl-PI-specific phospholipase (45) appears unlikely, however, because serum starvation (12–16 hr) did not result in their accumulation at the basolateral surface. On the other hand, the glycosyl-PI anchor might undergo a selective modification resulting in resistance to cleavage by PI-PLC. One such covalent modification of glycosyl-PI has been detected for acetylcholinesterase of the human erythrocyte, in which the inositol ring is acylated (40).

In conclusion, our results suggest the exciting possibility that a glycolipid, glycosyl-PI may function as a targeting signal for a specific class of apical membrane proteins in epithelial cells. Recent work has shown that glycosphingolipids appear to be apically sorted in epithelial cells (25, 51, 52). Perhaps apical targeting of non-glycosyl-PI-linked glycoproteins might be carried out by noncovalent interactions with glycolipids. In this regard, some glycolipids appear to be ideally suited to act as "sorters," since above certain critical concentrations they segregate themselves into patches that exclude other components of the bilayer (53). Definitive proof of a role for glycosyl-PI in protein sorting must await a molecular biological approach (i.e., examining whether the signal sequence for glycosyl-PI attachment can target an unsorted or a basolateral antigen to the apical surface).

Note Added in Proof. We have recently used phenyl-Sepharose (addition of 200 μl of a 75% slurry per ml of sample; rotated for 20 hrs at 4°C), just prior to trichloroacetic acid precipitation, to drastically reduce the background caused by spontaneous release (without diminishing PI-PLC specific release). No glycosyl-PI-anchored proteins with a preferred basolateral distribution were detected under these conditions, confirming the preferred apical distribution of this class of membrane glycoproteins.

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