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Biophysical Chemistry 82 (1999) 121–127

Biophysical Chemistry

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# Possible roles of glycosphingolipids in lipid rafts

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Received 28 March 1999; accepted 15 September 1999

**Abstract**

Recent studies have suggested that glycosphingolipid (GSL)-cholesterol microdomains in cell membranes may function as platforms for the attachment of lipid-modified proteins, such as glycosylphosphatidylinositol (GPI)-anchored proteins and src-family tyrosine kinases. The microdomains are proposed to be involved in membrane trafficking of GPI-anchored proteins and in signal transduction via src-family kinases. Here, the possible roles of GSLs in the physical properties of these microdomains, as well as in membrane trafficking and signal transduction, are discussed. Sphingolipid depletion inhibits the intracellular transport of GPI-anchored proteins in biosynthetic traffic and endocytosis via GPI-anchored proteins. Antibodies against GSLs as well as GPI-anchored proteins co-precipitate src-family kinases. Antibody-mediated cross-linking of GSLs, as well as that of GPI-anchored proteins, induces a transient increase in the tyrosine phosphorylation of several substrates. Thus, GSLs have important roles in lipid rafts. 

*Keywords:* Lipid raft; Caveolae; Microdomain; Glycosphingolipid; src-family kinase; GPI-anchored protein

## 1. Introduction

Glycosphingolipids (GSLs) are components of the outer leaflet of the plasma membrane of all vertebrate cells, and may modulate the functions of membrane proteins by interacting with them. The species and the amounts of GSLs undergo profound changes during development and oncogenesis, suggesting that they may play a fundamental role in these processes [1,2]. However, the physiological role of GSLs remains obscure.

Recent studies suggest that GSLs, together with cholesterol, form microdomains, which are re-

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0301-4622/99/$ - see front matter 
PII: S0301-4622(99)00111-8

ferred to as lipid rafts or caveolae membranes [3–6]. Lipid rafts have been proposed to have roles in the transport of lipids and proteins as well as in signal transduction. These studies provide new insight into the functions of GSLs.

## 2. Lipid rafts

In epithelial cells, the plasma membranes are polarized into apical and basolateral domains. The apical domains are rich in GSLs and GPI-anchored proteins, a finding which led to the proposal that GSL clusters, or lipid rafts, are involved in the apical sorting of GPI-anchored proteins. Low-density, detergent-insoluble membrane fractions can be isolated from cells by sucrose density gradient centrifugation. These membrane fractions are rich in GSLs, cholesterol, GPI-anchored proteins, and signaling molecules such as src-family kinases [7]. This observation indicates the possible presence of lipid rafts in cells and their involvement in signal transduction. The presence of lipid rafts in vivo is supported by several lines of evidence, including the results of electron microscopy using the freeze-fracture technique [8], a single-particle tracking study [9], a fluorescence resonance energy transfer study [10], and a chemical crosslinking study [11]. Caveolae, which are flask-shaped invaginations of the plasma membrane that contain the marker protein caveolin, can also be isolated using similar methods and appear to contain many of the same components as rafts. Caveolae are thought to be built around rafts with caveolin.

The basic forces driving the formation of rafts are thought to be lipid interactions, which are weak and transient [4]. GSLs are relatively rich in saturated fatty acyl chains, which allow tight packing and confer the characteristic of a high melting temperature. On the other hand, phospholipids are relatively rich in *cis*-unsaturated fatty acyl chains (kinked structure), which prevent tight packing and confer the characteristic of a low melting temperature. Lipid rafts may exist as phase-separated domains in the membrane. Hydrogen bonding among oligosaccharides in GSLs is also thought to contribute to the formation of lipid rafts [3]. GPI-anchored proteins generally have saturated acyl chains, which are likely to be inserted preferentially into lipid rafts. Src-family kinases are modified by saturated-chain lipids: palmitoylation and myristoylation, which are likely to be inserted preferentially into lipid rafts. The lipid composition renders the rafts and their constituent proteins resistant to solubilization with non-ionic detergents. Fig. 1 shows a hypothetical model of a lipid raft [3,12].

The association of GSLs with the constituent proteins of lipid rafts, such as GPI-anchored proteins, src-family kinases, and caveolin, has also been shown by co-immunoprecipitation with an anti-GSL antibody [13–17].

## 3. Contribution of GSLs to the physical properties of lipid rafts

Inhibition of sphingolipid biosynthesis leads to increased solubility of GPI-anchored proteins in non-ionic detergents [18]. Single-particle tracking of a GPI-anchored protein and GSLs in a native membrane showed transient confinement in patches $\sim$300 nm in diameter, which are thought to represent lipid rafts in vivo. The size of the confining domain for the GPI-anchored protein is reduced by treatment with inhibitors of GSL biosynthesis [19]. These observations suggest that GSLs have an important role in raft formation.

## 4. GSL function in membrane trafficking by lipid rafts

Inhibition of sphingolipid synthesis reduces the rate of transport of GPI-anchored proteins in yeast, abolishes the polarized sorting of GPI-anchored proteins in epithelia, and inhibits endocytosis via GPI-anchored proteins in colon adenocarcinoma [20,21]. Exogenously administered GSL induces the internalization of the constituent proteins of lipid rafts, including GPI-anchored proteins [22]. These observations suggest that GSLs are necessary for membrane trafficking by rafts.

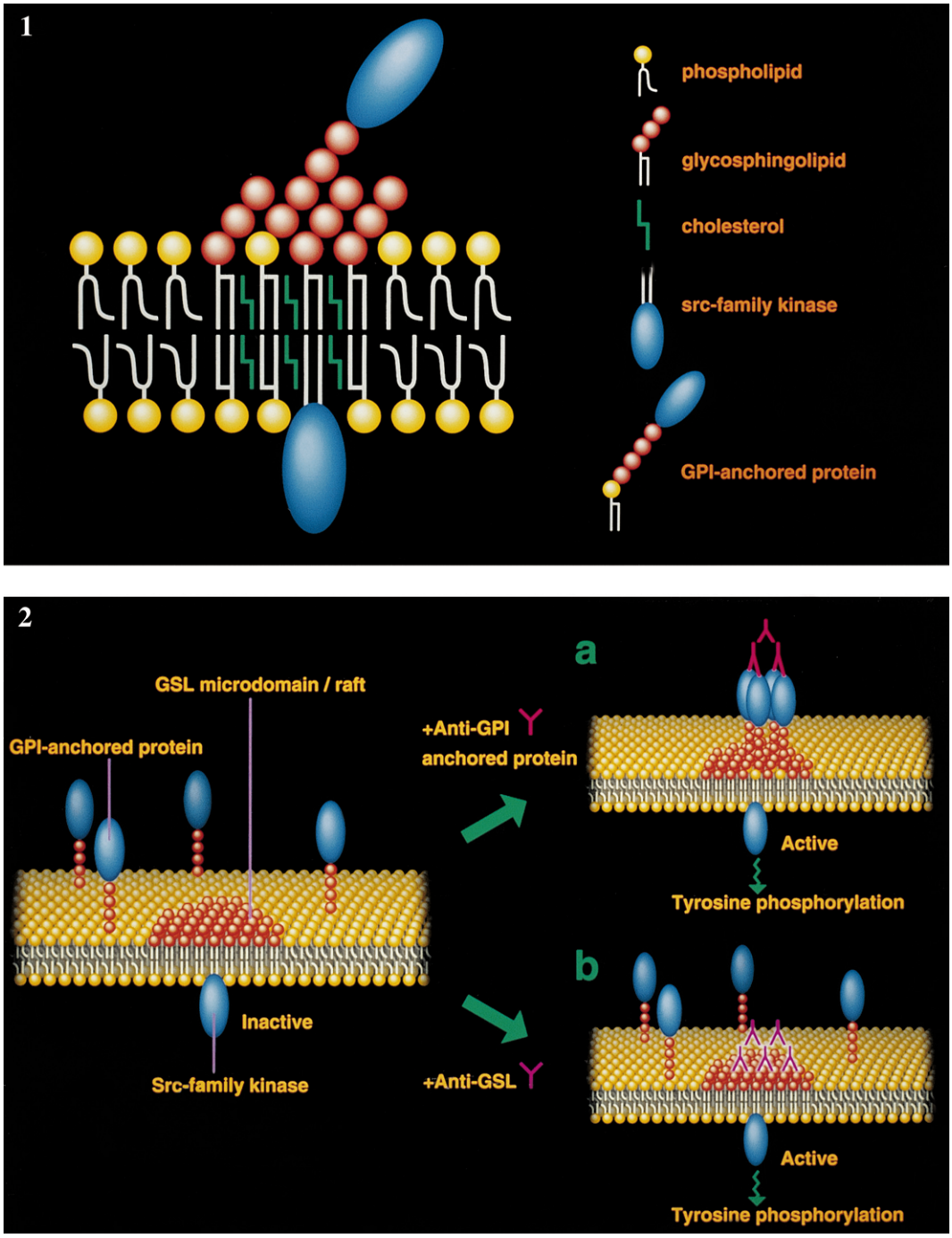


Fig. 1. Hypothetical model of a lipid raft.

Fig. 2. Activation of a signaling pathway via a src-family kinase by antibody-mediated crosslinking of: (a) GPI-anchored proteins or (b) GSL.

## 5. GSL functions in signal transduction via lipid rafts

Several GPI-anchored proteins have been implicated in transmembrane signaling via src-family tyrosine kinases [23]. In leukocytes, an anti-GPI-anchored protein antibody co-immunoprecipitates src-family kinases. Antibody-mediated crosslinking of GPI-anchored proteins induces transient tyrosine phosphorylation of several substrates, concomitantly with cell activation.

Interestingly, antibody-mediated crosslinking of GSLs can mimic GPI-anchored protein signaling [17,24]. GSL signaling and GPI-anchored protein signaling have many properties in common: (i) an anti-GSL antibody, as well as an anti-GPI-anchored protein antibody, co-immunoprecipitates src-family kinases; (ii) antibody-mediated crosslinking of GSLs as well as GPI-anchored proteins induces transient tyrosine phosphorylation of several substrates, including phospholipase C$\gamma$; (iii) both induce calcium flux; and (iv) both induce cell activation. These observations suggest that GSLs are involved in GPI-anchored protein signaling. Fig. 2 shows a schematic illustration of the signal transduction mediated by GSLs and GPI-anchored proteins [12]. Although there is some debate as to whether GPI-anchored proteins associate with lipid rafts in the steady state [11,25], it is thought that antibody-mediated crosslinking of GPI-anchored proteins induces their translocation to rafts or stabilizes their association with rafts. In support of this concept, we found that the association of lipid raft components with GPI-anchored proteins is weak or unstable in comparison with the association with GSLs in the steady state (unpublished observation).

Exogenously administered GSLs induce the activation of src-family kinases, the transient tyrosine phosphorylation of several substrates, and calcium flux [26]. Ligation of GSLs by bacterial toxins, such as the cholera toxin B subunit and the verotoxin B subunit, is known to induce signaling. GSL crosslinking or exogenously administered GSLs might induce a redistribution of signaling components, including src-family kinases, on the opposite cytoplasmic leaflet. However, the precise activation mechanism of the src-family kinases by GSLs is unknown.

Examples of the functions mediated by GSLs are listed in Table 1. It should be noted that most of these effects were induced by artificial stimulation, such as anti-GSL antibody treatment, etc. Several natural ligands for GSLs have been reported, such as selectin, gliolectin, galectin, sialoadhesin, and myelin-associated glycoprotein, suggesting possible signaling via the carbohydrate moieties of GSLs during cell adhesion [27]. However, the physiological significance of these molecules remains to be elucidated.

Lipid rafts are involved in signaling not only by GPI-anchored proteins but also by immunoreceptors, such as the T-cell receptor, the IgE receptor, and the growth factor receptor [5,22,28,29]. Leukocyte activation leads to the recruitment of these immunoreceptors and signaling molecules, including src-family kinases, to lipid rafts. Exogenously administered GSLs modulate T-cell receptor activation, IgE receptor-dependent histamine release, and the tyrosine kinase activity of the growth factor receptor [22,30,31]. Taken together, these observations suggest that GSLs have a significant role in signal transduction via lipid rafts.

## 6. GSL heterogeneity in lipid rafts

In melanoma, the ganglioside GM3 is recognized in the clustered form, but not the non-clustered form, as a melanoma-associated antigen [32] that is capable of adhering to endothelial cells [33], a process considered to initiate metastasis [34]. Recently, two membrane subfractions were separated from the low-density, detergent-insoluble fractions on a sucrose density gradient of the cells [35]. There is a clear difference in their lipid compositions and functions. The GM3-rich fraction, separated using the anti-GM3 antibody, contains sphingomyelin, cholesterol, and c-src, but not caveolin. In contrast, the caveolin-containing fraction, separated using the anti-caveolin antibody, contains glucosylceramide, a large quantity of cholesterol, and ras. The former is involved in GSL-dependent cell adhesion cou-

Table 1
Functional effects mediated by glycosphingolipids

| GSL | Agonist | Responding cell | Type of response | Ref |
|---|---|---|---|---|
| GM3 | Anti-GM3 | Mouse melanoma B16 | Tyrosine phosphorylation, growth inhibition | [39] |
| | | Neuro2A neuroblastoma | cAMP accumulation, differentiation | [40] |
| | Exogenous GM3 | Myelogenous leukemia HL60<br>Histiocytic lymphoma U937 | Monocytic differentiation | [41] |
| | | Myelogenous leukemia K562 | Megakaryocytoid differentiation | [42] |
| GD3 | Anti-GD3 | Human peripheral T cells | Tyrosine phosphorylation of PLCγ1<br>PI turnover, calcium flux<br>ras activation, proliferation | [43] |
| | | Primary cerebellar cells | Activation of src-family kinase tyrosine phosphorylation | [14] |
| | Exogenous GD3 | MDCK epithelial cells | Differentiation | [44] |
| | cDNA of GD3 synthase | Neuro2A neuroblastoma | Cholinergic differentiation | [45] |
| | | human cutaneous T lymphoma | Apoptosis | [46] |
| GM1 | Anti-GM1 | Rat thymocytes | Proliferation | [47] |
| | Cholera toxin B | Jurkat T cell line | Tyrosine phosphorylation of PLCγ1, calcium flux | [48] |
| | | Rat thymocytes | Calcium flux, proliferation | [49] |
| | | N18 neuroblastoma | Calcium flux, differentiation | [50] |
| | | Mouse 3T3 cells | Proliferation | [51] |
| | Exogenous GM1 | Jurkat T cell line | Activation of src-family kinase tyrosine phosphorylation of PLCγ1 | [26] |
| | | Neuro2A neuroblastoma | Calcium flux, differentiation | [52] |
| | | Primary neurons | Neurite outgrowth | [53] |
| GT1b | Exogenous GT1b | Human primary keratinocytes | Differentiation | [54] |
| GQ1b | Exogenous GQ1b | Mouse primary keratinocytes | PI turnover, calcium flux, differentiation | [55] |
| | | GOTO human neuroblastoma | Protein phosphorylation, differentiation | [56] |
| αGalGD1b | Anti-αGalGD1b | Basophilic leukemia RBL-2H3 | Activation of src-family kinase tyrosine phosphorylation of PLCγ1<br>PI turnover, calcium flux | [57] |
| NeuAcnLc | Exogenous NeuAcnLc | Myelogenous leukemia HL60 | Granulocytic differentiation | [58] |
| Gb3 | Anti-Gb3 verotoxin B | Burkitt's lymphoma cell line | Calcium flux, cAMP accumulation, apoptosis | [59] |

pled with signal transduction, whereas caveolae are not involved in cell adhesion. Thus, GSL heterogeneity exists in lipid rafts.

## 7. Possible role of GSLs in lipid rafts during development

The association of GPI-anchored proteins with lipid rafts is developmentally regulated during oligodendrocyte maturation [36]. Although GPI-anchored proteins are absent from the rafts fraction of a sucrose gradient of oligodendrocyte precursor cells, GPI-anchored proteins from maturing oligodendrocytes and myelin are present. Interestingly, a functional breakdown of the lipid bilayer of the myelin membrane is observed in mice with a disruption in the synthesis of galactocerebroside, a major GSL in myelin [37,38].

Numerous experiments have shown that GSLs can modulate cell proliferation and differentiation (Table 1). GSL profiles are characteristic for cell lineages and stages of differentiation, and serve as useful markers of differentiation. For example, monocytic differentiation of the myelogenous leukemia cell line HL-60, by TPA treatment, induces upregulation of GM3 synthase, resulting in an increase in GM3. On the other hand, granulocytic differentiation, by retinoic acid treatment, induces upregulation of GSL synthases for NeuAcnLc, resulting in an increase in NeuAcnLc. Surprisingly, exogenously administered GM3 or NeuAcnLc induces monocytic or granulocytic differentiation, respectively. Expression of specific GSL synthases may play a critical role in regulating differentiation [60]. In support of this idea, transfection of the GSL synthase cDNA has been reported to also induce cell differentiation [45]. These observations suggest that the GSL changes during development may regulate cellular events by affecting raft function.

## 8. Pathological roles of GSL in lipid rafts

GSLs have been known to function as receptors for bacterial toxins. Cholera toxin and its receptor, ganglioside GM1, are present in lipid rafts [61]. Thus, lipid rafts are an entry site for bacterial toxins on the cell surface.

The amyloid β-protein is present in lipid rafts [62,63], which is probably due to its direct binding to gangliosides [64]. The ganglioside-mediated binding of the amyloid β-protein to the membrane induces a conformational change of the amyloid β-protein and accelerates the rate of β-amyloid fibril formation in vitro [65,66]. These observations suggest that the ganglioside-bound amyloid β-protein may act as a template that catalyzes fibrillogenesis in Alzheimer's disease.

## Acknowledgements

This work was supported by Grants-in-Aid (11121237 to K.K. and 10178102 to Y.S.) for Scientific Research on Priority Areas, and by a Grant-in-Aid (10780376 to K.K.) for Encouragement of Young Scientists, and also by a Grant-in-Aid (10480173 to Y.S.) for Scientific Research (B) from the Ministry of Education, Science, Sports and Culture of Japan. This work was also supported by the Yamada Science Foundation.

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