Review

How the molecular features of glycosphingolipids affect domain formation in fluid membranes

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ABSTRACT

Glycosphingolipids, sphingomyelin and cholesterol are often all found in the detergent resistant fraction of biological membranes and are therefore recognized as raft components, but they do not necessarily co-localize in the same lateral domains. From cell biological studies it is evident that different sphingolipid species can be found in different lateral regions within the same cellular membrane. Biophysical studies have shown that their tendency to co-localize with each other and with other membrane components is largely governed by structural features of all lipids present. Glycosphingolipids form gel-phase like domains in fluid lipid bilayers. Sphingomyelin readily associates with cholesterol, forming liquid-ordered phase domains, but glycosphingolipids do not readily form cholesterol-enriched domains by themselves. However, mixed sphingomyelin- and glycosphingolipid-rich domains appear to incorporate cholesterol. Recent studies indicate that the ceramide backbone structure as well as the number of sugar units and presence of charge in the glycosphingolipid head group will influence the partitioning of these lipids between lateral membrane domains. The properties of the domains will be largely influenced by the presence of glycosphingolipids, which have very high melting temperatures. The lateral partitioning of glycosphingolipid molecular species has only recently been studied more intensively, and a lot remains to be done in this field of research.

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Contents

1. Introduction

The current view of the raft concept of domains in biological membranes defines these ordered domains as enriched in glyco-sphingolipids (GSL), sphingomyelin (SM) and cholesterol, although their size and dynamics are still debated [1–3]. However, when looking at the membrane composition it is becoming increasingly evident that this is an oversimplification of the situation, since the lateral organization of membrane components is a much more complex process. Although this review focuses very specifically on the importance of glycosphingolipids we shall not forget the importance of proteins for formation of laterally segregated domains in biological membranes [4–6]. However, already in quite simple (two to three component) lipid bilayers the lateral distribution of each lipid component will be a complex function of the exact composition and temperature of the system. The lateral structure of membranes will also be influenced by physical phenomena like line-tension of domains, membrane curvature and mixing conditions. Therefore, when adding additional complexity to a membrane system, such as proteins and hundreds of different lipid species, we can only try to imagine the effects on the lateral organization of lipids in the membranes. When it comes to the large variety of sphingolipids the lateral partitioning of these has only recently been studied more intensively, and a lot remains to be done in this field of research.

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It has been clearly shown that SM accompanied by high enough concentrations of cholesterol will form liquid ordered (L_o) domains in fluid, phospholipid based model membranes (Fig. 1). These domains are proposed to be similar in their properties to the "rafts" in biological membranes [7–9]. Cholesterol is essential for formation of the L_o-phase [10,11]. However, gel-phase microdomains can be formed by saturated GSLs in bilayers even without cholesterol [12,13]. The propensity of sphingolipids in general to participate in lateral ordered domain formation stems from their saturated hydrophobic region in the naturally relevant species. Ordered domains can possibly also be stabilized by the hydrogen-bonding properties of sphingolipids at the membrane/water interface. Although GSLs, SM and cholesterol are all found in the detergent resistant fraction of biological membranes, it does not necessarily mean that these lipids co-localize in the same lateral domains (see Fig. 1 for a simple view of suggested domain arrangements). From cell biological studies it is evident that different sphingolipid species can be found in different lateral regions within the same cellular membrane [14–17]. In a study on raft-associated GPI-anchored proteins it was shown that there were significant differences in cerebroside content in distinct detergent resistant domains in the same natural membrane [18]. For GSLs in biological

membranes it has recently been noted that they form, not only "rafts", but also cholesterol independent glycosynapses (glycosignalling domains), which in addition to being lateral domains also participate in cell–cell interaction and recognition [19–22]. Already quite simple GSLs, like galactosylceramides (GalCer), have a tendency to induce interbilayer interactions [23]. A recent computer simulation also provided evidence for such interactions, specifically mediated by hydrogen bonding between hydroxyl groups from opposing GalCer molecules [24].

The structure of GSLs varies widely with very large differences in both acyl chain lengths and head group sizes and compositions. It is therefore easy to conclude that molecules which are so different in structure also behave differently when incorporated into membranes. Our recent finding that even palmitoyl glucosylceramide (GlcCer) and palmitoyl GalCer differ somewhat in their propensity to form and partition into ordered domains in bilayer membranes is indicative of how small changes in structure can influence the membrane behavior of these molecules significantly [25]. What drives the lateral segregation of GSLs and which features of these molecules determine their lateral distribution? It is an elaborate task to study the membrane behavior of all the different lipid species known today. However, it seems necessary, if we want to be able to understand biomembrane structure on the nano-level, to at least study representative species of some subpopulations of structurally similar species in model membranes. In this review we will summarize the latest findings in the model membrane field on the partitioning of GSLs between fluid and ordered membrane domains. Some excellent reviews from the last few years nicely summarize the earlier data in the field [17,21,26,27] and the reader is kindly referred to these and other review articles in this issue for more information.

Quite few studies have so far been published on the tendency of glycosphingolipids to form domains in complex model membranes. Techniques used to study lateral heterogeneity in phospholipid bilayers have often been found to work poorly for these structurally complex lipids and substantial methodological modifications are often needed before understandable results are obtained. Of the hundreds of different GSL species present in biological membranes, biophysical studies have mainly been performed with a few representative species. These include e.g., gangliosides GM3 and GM1, lactosylceramides (LacCer), sulfatides and the small neutral monoglycosylceramides (GlcCer and GalCer). The results gained from the studies in model membranes are a good starting point for understanding the complex biophysical behavior of these molecules. The general trend to automatically assume that all sphingolipids will co-localize with cholesterol in membrane domains is slowly fading as information on the effect of sphingolipid structure on their partitioning behavior and co-lipid interaction preferences is emerging.

2. Special structural features which distinguish GSLs from other membrane lipids

The structural features in common for the GSLs are the sphingoid base (mainly 18–20 carbons in length) and the long, mostly saturated amide-linked acyl chain. Natural GSLs also often contain hydroxylated acyl chains that will affect their membrane properties, usually by increasing the gel- to liquid-crystalline phase transition temperature (T_m) of the GSL due to an increased hydrogen bonding capacity [28,29]. The T_m for a glycosphingolipid is usually very high compared to a corresponding sphingomyelin or phosphatidylcholine, e.g., 85 °C for *N-palmitoyl*-GalCer (PGalCer) [30] compared to 41 °C for *N-palmitoyl*-sphingomyelin (PSM) [31,32]. Differential scanning calorimetry has shown that several long chain GSLs exhibit complex mesomorphic behavior with high chain melting temperatures and irreversible transitions between different stable and metastable gel phases [33–36]. Such behavior might originate from interdigitation,
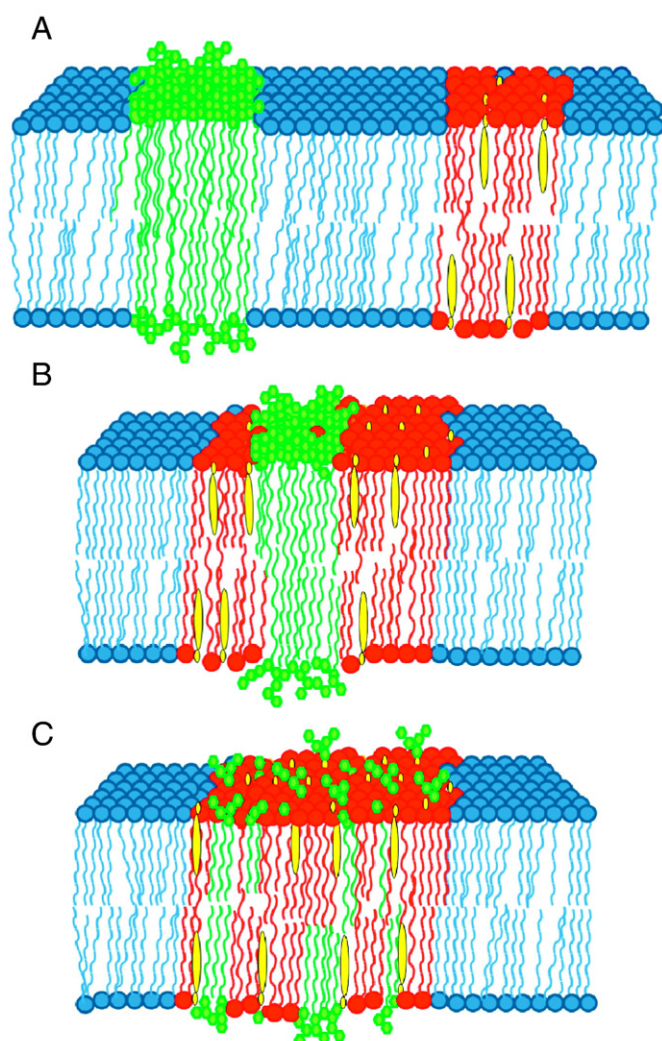

Fig. 1. Simplified picture of different possible domain arrangements in model bilayer membranes for glycosphingolipids (green), sphingomyelin (red) and cholesterol (yellow) in a fluid phospholipid (blue) bilayer. (A) Separate glycosphingolipid gel-phase domains and liquid-ordered domains formed by sphingomyelin and cholesterol. (B) Glycosphingolipid gel-phase domain within a liquid-ordered sphingomyelin/cholesterol domain. C) Mixed sphingolipid domain enriched in cholesterol.

i.e. the perturbation of an acyl chain originating in one monolayer into the other, as has been described for N-24:0-SM and for several neutral and charged very long chain GSLs with different degrees of head group complexity [37–40]. It has recently been suggested that such very long chain LacCer in human neutrophil membranes would function in stabilizing GSL enriched lateral membrane domains through interdigitation [41,42].

The structure of the polar head group may vary significantly, ranging from one neutral monosaccharide residue to big assemblies of carbohydrates and sialic acid, which gives the gangliosides their charged nature. Because of differences in the head group structure, the hydration of glycolipids is very different from that of phospholipids. For example as reported by computer simulations the interaction of water with oxygen atoms in GalCer (in a DPPG containing bilayer at 80 °C) resulted in a strong water ordering effect because of the formation of a spherical hydration shell around the head group and due to the formation of hydrogen bonds [24]. On average each GalCer made 8.6+/−0.1 hydrogen bonds with the surrounding water, and the carbohydrate head group was hydrated with about 10–11 water molecules. A substantial increase in the size of the head group was due to extensive hydrogen bonding to water. Therefore a large hydration shell, which increases with the number of sugar residues is seen for the glycosphingolipids [43–45]. Their membrane behavior is governed by a balance between the repulsions between these large head groups and the attractive interactions, involving intermolecular hydrogen bonding and hydrophobic interactions between the saturated hydrophobic parts of these molecules. Smaller GSLs have high T_m values and a tight lateral packing density in bilayer membranes [8,46,47], but the T_m will decrease for GSLs with larger head group structures [27]. For gangliosides it is evident that also the hydrophilic head groups can undergo rearrangements leading to cooperative changes between metastable phases in a ganglioside enriched bilayer [48].

As expected from their high T_m values GSLs tend to segregate from low T_m phospholipids in mixed membranes [49,50]. As a consequence GSL-enriched domains have a tight lateral packing density [47]. The lateral organization of acyl chain matched SMs and GalCers have been shown to differ when compared in monolayers at the air water interface [51–54]. GSLs with similar ceramide moieties have higher lateral packing density than corresponding SMs or glycerophospholipids [51,53,54]. The dense lipid packing seems to make cholesterol less miscible with GSLs compared to chain matched SMs at temperatures below the T_m of the sphingolipid [55]. Studies of the distribution of different GSLs in cellular membranes have mainly focused on the head group but have neglected to consider the importance of the acyl chain. It is, however, evident that the acyl structure will influence the membrane behavior of GSLs as well [56,57]. A few biophysical studies have taken the acyl chain composition into consideration and in combination with the knowledge of ceramide structure in naturally occurring GSLs some information on how acyl chain composition affects the partitioning between lateral membrane regions can be gained. It has been shown by fluorescence spectroscopy and microscopy that the partitioning of several different GSLs between domains in different phase states in model membranes is largely governed by the acyl chain length [13,58]. However, general rules for predicting GSL partitioning between membrane domains are hard to find and, as will be discussed below, this becomes even harder with increasing complexity in the structure of the head group.

3. Small neutral GSLs segregate in fluid membranes

The thermotropic behavior of small neutral GSLs has in general been shown to be very complex [52,59–61]. The phase behavior of GalCer and GlcCer with varying acyl chains has been summarized by Koynova and Caffrey in 1995 [62] and LacCers were recently discussed by Li et al. [52]. The chain-melting phase transition temperatures of these GSLs are usually a lot higher than for biologically relevant SM and glycerophospholipids [62].

The possible co-localization of some acyl chain defined GSLs and cholesterol in the same ordered domains in mixed bilayer membranes was recently explored in a fluorescence quenching study. The GSLs studied were GalCer, GlcCer and LacCer with a palmitic acid in the N-linked position (PGalCer, PGlcCer and PLacCer, respectively) [25]. The formation of sterol-enriched domains in a fluid bilayer was examined using cholestatrienol (CTL) as a fluorescent reporter molecule, selective for sterol-enriched domains, together with a quencher in the disordered phase. Quenching data for another probe (trans-parinaric acid, tPA) showed that ordered domains were present initially in the PGalCer, PGlcCer and PLacCer containing bilayers. The stereochemical orientation of one hydroxyl group in the sugar moiety, as seen when comparing GalCer and GlcCer, affected their domain forming properties. When no other sphingolipids were present in the bilayers, GalCer (at 30 mol% bilayer concentration) formed ordered domains which dissociated with increasing temperature in a complicated two step process. GlcCer at the same concentration was shown to form domains with a more cooperative dissociation behavior, but lower thermostability. The saturated GSLs, which have very high T_m-values, were probably forming gel-phase domains in the fluid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) matrix of the mixed lipid bilayers [12,55].

Computer simulations on glyceroglycolipids comparing glucose versus galactose derivatives have also revealed differences in their behavior [63]. When the order parameter (S_{mol} of carbons 4–16) of the acyl chains were examined at 70 °C, both dipalmitoyl galactosylglycerol and dipamitoyl glucosylglycerol had more ordered acyl chains (both sn-1 and sn-2) than dipalmitoyl phosphatidylcholine at the same temperature. The orientation of the carbohydrate head group in the two glycoglycerolipids was similar, but it was noticed that the affinity of the galactose head group to participate in hydrogen bonding was higher than that of the glucose head group. The galactose hydrogen bonding was mostly between lipids, whereas the simulations indicated that glucose hydrogen bonded more to water than galactose. This is also in line with previous physicochemical studies which have indicated that galactoglycerolipids confer higher stability to membrane phases than the corresponding glucoglycerolipids [64,65].

The domain forming behavior of LacCer showed that the size of the head group was not the major determinant for the capacity of the GSLs to form ordered domains as it behaved quite similarly to the monoglycosylceramides [25]. It was also shown that pure LacCer or GlcCer can form lateral domains that contain some cholesterol, whereas those formed by pure GalCer largely exclude the sterol [25]. The conclusion was drawn that the strong cohesion between PGalCer molecules leads to packing constraints that hindered the partitioning of sterol into these domains. Cholesterol also has only moderate effects on domain formation by mixed brain GalCers [66] and solid phase immiscibility of PGalCer with cholesterol at temperatures below the T_m of the glycolipid has been reported [55]. However, a contradictory study using vibrational infrared spectroscopy to look at GalCer/cholesterol/1,2-di-palmitoyl-sn-glycero-3-phosphocholine (DPPC) mixtures, in which the formation of small domains was clearly seen, indicated that cholesterol preferred to interact with GalCer over interacting with DPPC both above and below the T_m of both lipids in this highly saturated bilayer system [67]. An extensive overview of the behavior of small neutral GSLs and SMs with cholesterol in monolayers was presented in 2006 by Brown and colleagues [54].

Recent studies on supported lipid bilayers and giant unilamellar vesicles (GUVs) containing GalCer (with mixed acyl chains), cholesterol and various fluid phase phospholipids imaged by atomic force microscopy (AFM) and fluorescence microscopy, respectively, have revealed some details about the structure of domains in such systems [68,69]. In the supported lipid bilayers the GalCer seemed to be

distributed to the leaflet distal to the mica substrate rendering these membranes asymmetric. The results from GUVs were comparable with those from the supported lipid bilayers although the GalCer domains in the GUVs were symmetric, with GalCer in both leaflets. It was found that mixtures of 1,2-di-lauroyl-sn-glycero-3-phosphocholine (DLPC)/GalCer/cholesterol at varying concentrations displayed only gel-fluid co-existence, indicating that with this particular glycerophospholipid, cholesterol was excluded from the GalCer-rich domains [68]. In the second study samples contained 65 mol% 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), POPC or DLPC as the fluid component. Here it was apparent that the interactions between cholesterol and GalCer in three component membranes were largely dependent on the fluid phase component [69]. It seems from these studies that GalCer and cholesterol can form L_o-phase domains in membranes where the interaction of cholesterol with the fluid phase component is disfavored. According to these results, sterol partitioning between domains will be influenced by length mismatch with the fluid lipid in the hydrophobic region as well as by unsaturation of the acyl chains and by cholesterol concentration in the bilayer [69]. The acyl chain length also seems to be of importance for the distribution of GalCer between different lateral domains in biological membranes [70].

SMs have been shown to form sterol-rich domains in mixed bilayer membranes [71]. When neutral GSLs were incorporated into mixed bilayers with POPC, SM and cholesterol the fluorescence quenching results obtained both with CTL and tPA as the fluorescent probes showed that GalCer, GlcCer and LacCer all associated with and stabilized the (PSM)/sterol domains [25]. The neutral GSLs were in these studies added in equimolar amounts to PSM. Unlike ceramides, these GSLs do not exclude/displace sterols from SM/cholesterol domains [72,73], but rather associate with these domains making them more stable against temperature induced dissociation [25]. None of the mono- and diglycosylceramides studied so far has been shown to be very prone to associate with cholesterol when present as the only high-T_m lipid in the bilayer. The presence of a SM in the same membrane favored the association of the GSLs with cholesterol. The observed stabilizing effect on PSM/sterol domains by GSLs suggests that they in turn may favor the formation of sterol-enriched domains in biological systems. However, there are yet no indications that any other sphingo- or phospholipid could interact with cholesterol and form liquid-ordered phase domains as well as SM does.

The above mentioned findings regarding their membrane properties might be implicated in the sorting of small neutral GSLs between subcellular membranes, knowing that they follow different biosynthetic and sorting pathways in biological systems [74,75].

4. The effect of charge in the GSL head group on lateral domain formation

Many biologically relevant GSLs have charged sugar head groups. Here we have chosen to take sulfatide (galactosylceramidesulfate) as an example of how a charge in the head group of a GSL might affect the membrane behavior. We have chosen sulfatide for three reasons 1) it is a fairly abundant lipid in biological membranes, 2) it has been studied in model membranes to quite a large extent compared to other charged GSLs and 3) the results from such studies are not complicated by the effect of large head group structures as in some other charged GSLs, like the gangliosides.

Sulfatides are negatively charged GSLs, which are minor constituents of most eukaryotic membranes but more abundant in the nervous system and especially important in myelin membranes [76]. In many cells sulfatides have been found to function as receptors for neurotransmitters, opiates, endorphins and a heat shock protein, Hsp70 [77–83]. In the myelin membranes sulfatides take part in contact formation between bilayers through interaction with GalCer [84,85]. This interaction is of crucial importance for the stability of the myelin in the central nervous system and the myelin membranes are

also highly enriched in both GalCer and cholesterol [85]. Sulfatides are found in the detergent insoluble fraction of the myelin membranes, which could suggest that they take part in lateral domain formation [86,87].

Model membrane studies have shown that sulfatides are protected against antibody recognition in a SM/cholesterol environment compared to a phosphatidylcholine/cholesterol environment [88], suggesting significant differences in the interaction with sphingolipid versus glycerophospholipid rich membranes. A recent study used AFM on supported lipid bilayers to look at the partitioning of sulfoglycolipids in membranes compositionally resembling those of the sperm plasma membrane [89]. The results showed that sulfatide was localized in ordered domains in the supported bilayers. Studies on the membrane properties of sulfatides have often been conducted on biological mixtures of sulfatides with a wide variety of acyl chains of different lengths and degree of hydroxylation [89,90]. It is quite clear, as for the other GSLs, that the ceramide part of the sulfatide molecule will influence their membrane properties [28,91]. The thermotropic behavior of pure acyl chain defined sulfatides has been extensively studied by Boggs and co-workers [28,92–94]. N-palmitoyl-sulfatide has a transition temperature significantly lower than that of the corresponding non-sulphated GalCer [28,95]. N-palmitoyl-sulfatide was found to occur in two different gel phases of different stability and the complex thermotropic behavior was highly dependent on the hydration level [95]. A recent study of N-palmitoyl-sulfatide in mixed bilayer membranes showed that this sulfatide at low, physiologically relevant, concentrations readily partitioned into ordered domains together with other sphingolipids, such as SM and GalCer, and cholesterol [96]. Therefore, it can be concluded that sulfatide is most likely to participate in lateral domain formation with other sphingolipids also in biological membranes. However, when present as the only sphingolipid in the bilayer the charge repulsions between sulfatide molecules made them form ordered domains that were very unstable against temperature compared to domains formed by a corresponding GalCer at the same bilayer concentration [96].

The membrane properties of sulfatides have also been shown to be dependent on divalent cations, such as calcium [28,78,97]. The thermotropic behavior of sulfatide is dependent on the presence of counter ions in a complex and acyl chain dependent way [28]. In the presence of calcium a condensing effect on a pure sulfatide monolayer is detected, indicating a reduction in repulsive forces or an increase in attractive intermolecular interactions between sulfatide molecules within the plane of the membrane [98]. In bilayers composed of a saturated phosphatidylcholine mixed with sulfatide the phase transition temperature is increased in the presence of mM concentrations of calcium as determined by diphenylhexatriene (DPH) anisotropy [78]. Similarly to GalCer, also sulfatide has been shown to form an intermolecular hydrogen bonding network in the head group region [99,100]. According to Fourier transform infrared spectroscopy (FTIR) the amount of inter- and intramolecular hydrogen bonds of the head group are reduced within pure sulfatide bilayers when calcium binds to the sulphate [97,99]. The binding causes molecular rearrangements in the head group region so that the bilayer properties of the molecules are significantly changed [97]. Calcium has also been found to stabilize the intermembrane carbohydrate-carbohydrate interactions between GalCer and sulfatide [101–103]. The thermostability of sphingolipid domains containing N-palmitoyl-sulfatide will also be increased by the presence of divalent counter ions, such as calcium [96].

5. The complex domain behavior of gangliosides

Gangliosides are structurally more complex than the GSLs discussed so far in this review. The segregation of these complex GSLs into domains in biological membranes is a well established fact [5,17,21,26]. This is a cholesterol-independent process since ganglio-

sides can clearly segregate from other lipids both in the presence and in the absence of cholesterol [104–107]. As noted by Sonnino et al. in a review from 2006 [26] the special physicochemical properties of these GSLs seem to be very important, if not even crucial, for the properties of membrane domains in biological membranes. The cell biological studies have however given controversial results regarding the distribution of different gangliosides between lateral domains and consensus on this point is apparently hard to reach. It is especially important to keep in mind that glycosphingolipids are found almost exclusively in the outer leaflet of the plasma membranes of cells. This asymmetric distribution is often lost in the model membrane studies discussed more extensively in this review. Long acyl chains in combination with an asymmetric distribution might lead to interdigitation of acyl chains into the opposing leaflet of the bilayer [38–40,108]. The effects of such interdigitation on domain formation in the opposing membrane leaflets is yet largely unknown. It also seems evident that a redistribution of gangliosides, as well as other, membrane components continuously happens during the life cycle of a cell and that the distribution therefore might be hard to predict. It is, however, becoming increasingly evident that GSLs are active components in cellular events and that the redistribution of these membrane components within the membrane plane according to certain stimuli might be of crucial importance for signalling events at the cell surface. A major challenge for the biophysicists in the field is now to correlate the distribution of membrane components with these cellular events and to study model systems that mimic snapshots of a cellular membrane at a time point of interest.

The phase location of components of myelin membranes was recently reported by immunolabelling of these complex lipid-protein mixtures after Langmuir–Schaeffer film transfer to silanized coverslips [109]. The results showed that ganglioside GM1 in such mixtures will be located in the liquid-expanded phase, whereas GalCer and cholesterol will be found predominantly in the L_o-phase domains. Another type of segregation has been seen with ganglioside GM1 in a SM based bilayer, where GM1 ganglioside and cholesterol partitioned into different domains, one sterol-SM domain and one GM1-SM domain [110]. GM1 can be inserted into one leaflet of supported lipid bilayers by spontaneous transfer of up to 30% GM1 from a micellar aqueous solution [111]. A study using this technique measured the diffusion coefficients of Texas Red labeled 1,2-di-palmitoyl-*sn*-glycero-3-phosphoethanolamine (TR-DPPE) in samples of 1,2-di-oleoyl-*sn*-glycero-3-phosphocholine (DOPC)/brain-SM/cholesterol with and without GM1 through continuous bleaching. The average diffusion coefficient for the probe in the bilayer was reduced by the addition of GM1 by more than 50% [111]. Since TR-DPPE partitions preferentially into the less ordered phase when phase coexistence occurs [112], these results suggest that the addition of GM1 to a sample containing 20 mol% cholesterol, in which there is fluid phase separation, leads to substantial rearrangements of the liquid-crystalline phase where the diffusion was reduced by 75%. From these studies it is evident that the partitioning of a ganglioside like GM1 might vary significantly depending on the surrounding lipids.

A monolayer study characterizing the interaction of GM3 ganglioside with POPC versus SM shows that this GSL has no direct preference for either one of these bulk lipids [113]. The same investigators have also reported that the cholesterol desorption from lipid monolayers to β-cyclodextrin in the subphase is comparable from GM3 ganglioside and POPC monolayers, whereas it is much slower from monolayers composed of SM, indicating a stronger interaction between cholesterol and the latter [114].

Studies like those mentioned above indicate that GM1 and cholesterol would not readily co-localize. However, it has been known for a long time already that GM1 and cholesterol can be enriched in the same membrane domains, since these lipids are both found in the caveolae [115,116]. In epithelial cells it has also been shown that GM1, but not GM3, co-localized in the same domains with

cholesterol [14]. The varying results on co-localization of different GSLs and of GSLs and cholesterol in cellular membranes can depend on the methods used to detect them, but it might also be that cells at different stages of differentiation or during different cellular processes have their GSLs organized differently between lateral membrane domains as indicated for example by studies on T-cells where reorganization of GM1 and GM3 has been indicated during polarization [117].

One explanation for the varying results on GM1 partitioning might be as Wang and Silvius concluded in 2003, that the partitioning of gangliosides, like other GSLs, between different phases is largely governed by the acyl chain composition, rather than by the head group [13]. A study on the distribution of ganglioside GM1 between gel- and fluid phases in two-component phosphatidylcholine membranes has actually also shown that the preference of this ganglioside for the so-called ripple-phase decreased with decreasing acyl chain length or with increasing unsaturation of the chain [118]. However, it has also been suggested that the partitioning of gangliosides between domains will be dependent on the number of sugar residues in the head group [26].

Clusters of GM1-rich domains within gel-phase domains in phosphatidylcholine monolayers have been reported by AFM on monolayers on solid support [119,120]. A recent monolayer study on DPPC/GM1- mixtures focused on answering the question how GM1, which displays liquid-expanded isotherms in surface monolayers can come to reside in ordered domains in more complex lipid mixtures [121]. It was found that the ganglioside condenses the DPPC monolayer at concentrations lower than 25 mol% of ganglioside. A model for a 3:1 DPPC:ganglioside geometric complex was proposed, which could explain the formation of different kinds of domains in this system when studied by AFM. This study indicates that there is a different condensed phase formed by these stoichiometric complexes in co-existence with DPPC condensed domains and DPPC liquid-expanded domains in the monolayer. These results are supported by computational simulations on GM1 effects on DPPC bilayers [122,123]. The addition of up to 25 mol% GM1 to a DPPC bilayer at 52 °C was observed to lead to a condensing effect on the DPPC molecular area. The addition of GM1 to a DPPC bilayer was also shown to increase the simulated deuterium order parameter of the hydrocarbon chains order profile, a result which is consistent with the GM1 induced condensing on DPPC molecular area seen both in the simulations and in the monolayer study mentioned above [121–123]. These results indicate that gangliosides might form ordered domains or complexes within ordered domains formed by other membrane lipids. Such domains may with many techniques be hard to distinguish from each other. In line with these findings GM1 was recently shown to be enriched in the ceramide rich domains in supported lipid bilayers showing three-phase separation into liquid disordered, liquid ordered and ceramide rich domains [124].

6. Conclusions

GSLs segregate in biological and model membranes forming gel-phase-like domains, which may be incorporated into other ordered domains or exist as separate domains in the bulk fluid bilayer (see Fig. 1). The partitioning of different GSL species between lateral domains seems to be a complicated function of their structural features including both polar head group and ceramide backbone. Although SM readily associates with cholesterol, forming L_o-phase domains, the evidence indicates that GSLs do not readily form cholesterol-enriched domains by themselves. However, mixed SM- and GSL-rich domains appear to incorporate cholesterol. This might explain how all of these components can be found in the same domains in biological membranes. However, we should remember that in cellular membranes GSL–protein interactions probably influence the lateral segregation of these components.

Acknowledgements

References

[1] D.A. Brown, E. London, Structure of detergent-resistant membrane domains: does phase separation occur in biological membranes? Biochem. Biophys. Res. Commun. 240 (1997) 1–7.

[2] L.J. Pike, Rafts defined: a report on the Keystone Symposium on Lipid Rafts and Cell Function, J. Lipid. Res. 47 (2006) 1597–1598.

[3] K. Simons, E. Ikonen, Functional rafts in cell membranes, Nature 387 (1997) 569–572.

[4] R.F. Epand, B.G. Sayer, R.M. Epand, Induction of raft-like domains by a myristoylated NAP-22 peptide and its Tyr mutant, FEBS J. 272 (2005) 1792–1803.

[5] G. van Meer, D.R. Voelker, G.W. Feigenson, Membrane lipids: where they are and how they behave, Nat. Rev., Mol. Cell Biol. 9 (2008) 112–124.

[6] R.M. Epand, Proteins and cholesterol-rich domains, Biochim. Biophys. Acta 1778 (2008) 1576–1582.

[7] M. Ge, K.A. Field, R. Aneja, D. Holowka, B. Baird, J.H. Freed, Electron spin resonance characterization of liquid ordered phase of detergent-resistant membranes from RBL-2H3 cells, Biophys. J. 77 (1999) 925–933.

[8] R. Schroeder, E. London, D. Brown, Interactions between saturated acyl chains confer detergent resistance on lipids and glycosylphosphatidylinositol (GPI)-anchored proteins: GPI-anchored proteins in liposomes and cells show similar behavior, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 12130–12134.

[9] K. Simons, E. Ikonen, How cells handle cholesterol, Science 290 (2000) 1721–1726.

[10] D.A. Brown, E. London, Structure and function of sphingolipid- and cholesterol-rich membrane rafts, J. Biol. Chem. 275 (2000) 17221–17224.

[11] J.H. Ipsen, G. Karlstrom, O.G. Mouritsen, H. Wennerstrom, M.J. Zuckermann, Phase equilibria in the phosphatidylcholine-cholesterol system, Biochim. Biophys. Acta 905 (1987) 162–172.

[12] T.E. Thompson, T.W. Tillack, Organization of glycosphingolipids in bilayers and plasma membranes of mammalian cells, Ann. Rev. Biophys. Biophys. Chem. 14 (1985) 361–386.

[13] T.Y. Wang, J.R. Silvius, Sphingolipid partitioning into ordered domains in cholesterol-free and cholesterol-containing lipid bilayers, Biophys. J. 84 (2003) 367–378.

[14] P. Janich, D. Corbeil, GM1 and GM3 gangliosides highlight distinct lipid micro-domains within the apical domain of epithelial cells, FEBS Lett. 581 (2007) 1783–1787.

[15] V. Chigorno, P. Palestini, M. Sciannamblo, V. Dolo, A. Pavan, G. Tettamanti, S. Sonnino, Evidence that ganglioside enriched domains are distinct from caveolae in MDCK II and human fibroblast cells in culture, Eur. J. Biochem. 267 (2000) 4187–4197.

[16] K. Iwabuchi, K. Handa, S. Hakomori, Separation of "glycosphingolipid signaling domain" from caveolin-containing membrane fraction in mouse melanoma B16 cells and its role in cell adhesion coupled with signaling, J. Biol. Chem. 273 (1998) 33766–33773.

[17] S. Hoetzl, H. Sprong, M.G. van, The way we view cellular (glyco)sphingolipids, J. Neurochem. 103 (Suppl 1) (2007) 3–13.

[18] B. Brugger, C. Graham, I. Leibrecht, E. Mombelli, A. Jen, F. Wieland, R. Morris, The membrane domains occupied by glycosylphosphatidylinositol-anchored prion protein and Thy-1 differ in lipid composition, J. Biol. Chem. 279 (2004) 7530–7536.

[19] S. Hakomori, K. Handa, Glycosphingolipid-dependent cross-talk between glyco-synapses interfacing tumor cells with their host cells: essential basis to define tumor malignancy, FEBS Lett. 531 (2002) 88–92.

[20] S. Hakomori, Glycosynapses: microdomains controlling carbohydrate-dependent cell adhesion and signaling, An. Acad. Bras. Cienc. 76 (2004) 553–572.

[21] S.I. Hakomori, Structure and function of glycosphingolipids and sphingolipids: recollections and future trends, Biochim. Biophys. Acta 1780 (2008) 325–346.

[22] F. Pincet, B.T. Le, Y. Zhang, J. Esnault, J.M. Mallet, E. Perez, P. Sinay, Ultraweak sugar-sugar interactions for transient cell adhesion, Biophys. J. 80 (2001) 1354–1358.

[23] K. Kulkarni, D.S. Snyder, T.J. McIntosh, Adhesion between cerebroside bilayers, Biochemistry 38 (1999) 15264–15271.

[24] T. Zaraiskaya, K.R. Jeffrey, Molecular dynamics simulations and 2H NMR study of the GalCer/DPPG lipid bilayer, Biophys. J. 88 (2005) 4017–4031.

[25] S. Maunula, Y.J. Bjorkqvist, J.P. Slotte, B. Ramstedt, Differences in the domain forming properties of N-palmitoylated neutral glycosphingolipids in bilayer membranes, Biochim. Biophys. Acta 1768 (2007) 336–345.

[26] S. Sonnino, A. Prinetti, L. Mauri, V. Chigorno, G. Tettamanti, Dynamic and structural properties of sphingolipids as driving forces for the formation of membrane domains, Chem. Rev. 106 (2006) 2111–2125.

[27] B. Maggio, M.L. Fanani, C.M. Rosetti, N. Wilke, Biophysics of sphingolipids II. Glycosphingolipids: an assortment of multiple structural information transducers at the membrane surface, Biochim. Biophys. Acta 1758 (2006) 1922–1944.

[28] J.M. Boggs, K.M. Koshy, G. Rangaraj, Effect of fatty acid chain length, fatty acid

[29] hydroxylation, and various cations on phase behavior of synthetic cerebroside sulfate, Chem. Phys. Lipids 36 (1984) 65–89.

[29] K.M. Koshy, J.M. Boggs, Partial synthesis and physical properties of cerebroside sulfate containing palmitic acid or alpha-hydroxy palmitic acid, Chem. Phys. Lipids. 34 (1983) 41–53.

[30] M.J. Ruocco, D. Atkinson, D.M. Small, R.P. Skarjune, E. Oldfield, G.G. Shipley, X-ray diffraction and calorimetric study of anhydrous and hydrated N-palmitoylga-lactosylsphingosine (cerebroside), Biochemistry 20 (1981) 5957–5966.

[31] L.K. Bar, Y. Barenholz, T.E. Thompson, Effect of sphingomyelin composition on the phase structure of phosphatidylcholine-sphingomyelin bilayers, Biochemistry 36 (1997) 2507–2516.

[32] B. Ramstedt, J.P. Slotte, Comparison of the biophysical properties of racemic and D-erythro-N-acyl sphingomyelins, Biophys. J. 77 (1999) 1498–1506.

[33] W. Curatolo, F.B. Jungalwala, Phase behavior of galactocerebrosides from bovine brain, Biochemistry 24 (1985) 6608–6613.

[34] V.S. Kulkarni, W.H. Anderson, R.E. Brown, Bilayer nanotubes and helical ribbons formed by hydrated galactosylceramides: acyl chain and headgroup effects, Biophys. J. 69 (1995) 1976–1986.

[35] R.A. Reed, G.G. Shipley, Structure and metastability of N-lignocerylgalac-tosylsphingosine (cerebroside) bilayers, Biochim. Biophys. Acta 896 (1987) 153–164.

[36] E. Freire, D. Bach, M. Correa-Freire, I. Miller, Y. Barenholz, Calorimetric investigation of the complex phase behavior of glucocerebroside dispersions, Biochemistry 19 (1980) 3662–3665.

[37] I.E. Mehlhorn, E. Florio, K.R. Barber, C. Lordo, C.W. Grant, Evidence that trans-bilayer interdigitation of glycosphingolipid long chain fatty acids may be a general phenomenon, Biochim. Biophys. Acta 939 (1988) 151–159.

[38] I.W. Levin, T.E. Thompson, Y. Barenholz, C. Huang, Two types of hydro-carbon chain interdigitation in sphingomyelin bilayers, Biochemistry 24 (1985) 6282–6286.

[39] P.R. Maulik, D. Atkinson, G.G. Shipley, X-ray scattering of vesicles of N-acyl sphingomyelins. Determination of bilayer thickness, Biophys. J. 50 (1986) 1071–1077.

[40] J.M. Boggs, K.M. Koshy, Do the long fatty acid chains of sphingolipids interdigitate across the center of a bilayer of shorter chain symmetric phospholipids? Biochim. Biophys. Acta 1189 (1994) 233–241.

[41] K. Iwabuchi, A. Prinetti, S. Sonnino, L. Mauri, T. Kobayashi, K. Ishii, N. Kaga, K. Murayama, H. Kurihara, H. Nakayama, F. Yoshizaki, K. Takamori, H. Ogawa, I. Nagaoka, Involvement of very long fatty acid-containing lactosylceramide in lactosylceramide-mediated superoxide generation and migration in neutrophils, Glycoconj. J. 25 (2008) 357–374.

[42] F. Yoshizaki, H. Nakayama, C. Iwahara, K. Takamori, H. Ogawa, K. Iwabuchi, Role of glycosphingolipid-enriched microdomains in innate immunity: microdomain-dependent phagocytic cell functions, Biochim. Biophys. Acta 1780 (2008) 383–392.

[43] C. Arnulphi, P.R. Levstein, M.E. Ramia, C.A. Martin, G.D. Fidelio, Ganglioside hydration study by 2H-NMR: dependence on temperature and water/lipid ratio, J. Lipid Res. 38 (1997) 1412–1420.

[44] C. Arnulphi, C.A. Martin, G.D. Fidelio, Mixed lipid aggregates containing ganglio-sides impose different 2H-NMR dynamical parameters on water environment depending on their lipid composition, Mol. Membr. Biol. 20 (2003) 319–327.

[45] D. Bach, B. Sela, I.R. Miller, Compositional aspects of lipid hydration, Chem. Phys. Lipids 31 (1982) 381–394.

[46] R.E. Brown, Sphingolipid organization in biomembranes: what physical studies of model membranes reveal, J. Cell. Sci. 111 (1998) 1–9.

[47] M. Masserini, D. Ravasi, Role of sphingolipids in the biogenesis of membrane domains, Biochim. Biophys. Acta 1532 (2001) 149–161.

[48] L. Cantu, M. Corti, F.E. Del, E. Digirolamo, S. Sonnino, G. Tettamanti, Experimental evidence of a temperature-related conformational change of the hydrophilic portion of gangliosides, Chem. Phys. Lipids 79 (1996) 137–145.

[49] M. Gardam, J.R. Silvius, Intermixing of dipalmitoylphosphatidylcholine with phospho- and sphingolipids bearing highly asymmetric hydrocarbon chains, Biochim. Biophys. Acta 980 (1989) 319–325.

[50] M.R. Morrow, D. Singh, D. Lu, C.W. Grant, Glycosphingolipid phase behaviour in unsaturated phosphatidylcholine bilayers: a 2H-NMR study, Biochim. Biophys. Acta 1106 (1992) 85–93.

[51] X.M. Li, J.M. Smaby, M.M. Momsen, H.L. Brockman, R.E. Brown, Sphingomyelin interfacial behavior: the impact of changing acyl chain composition, Biophys. J. 78 (2000) 1921–1931.

[52] X.M. Li, M.M. Momsen, H.L. Brockman, R.E. Brown, Lactosylceramide: effect of acyl chain structure on phase behavior and molecular packing, Biophys. J. 83 (2002) 1535–1546.

[53] J.M. Smaby, V.S. Kulkarni, M. Momsen, R.E. Brown, The interfacial elastic packing interactions of galactosylceramides, sphingomyelins, and phosphatidylcholines, Biophys. J. 70 (1996) 868–877.

[54] X. Zhai, X.M. Li, M.M. Momsen, H.L. Brockman, R.E. Brown, Lactosylceramide: lateral interactions with cholesterol, Biophys. J. 91 (2006) 2490–2500.

[55] M.J. Ruocco, G.G. Shipley, Interaction of cholesterol with galactocerebroside and galactocerebroside-phosphatidylcholine bilayer membranes, Biophys. J. 46 (1984) 695–707.

[56] S. Ali, H.L. Brockman, R.E. Brown, Structural determinants of miscibility in surface films of galactosylceramide and phosphatidylcholine: effect of unsaturation in the galactosylceramide acyl chain, Biochemistry 30 (1991) 11198–11205.

[57] S. Ali, J.M. Smaby, R.E. Brown, Acyl structure regulates galactosylceramide's interfacial interactions, Biochemistry 32 (1993) 11696–11703.

[58] M. Koivusalo, J. Alvesalo, J.A. Virtanen, P. Somerharju, Partitioning of pyrene-

labeled phospho- and sphingolipids between ordered and disordered bilayer domains, Biophys. J. 86 (2004) 923–935.

[59] V.S. Kulkarni, R.E. Brown, Thermotropic behavior of galactosylceramides with cis-monoenoic fatty acyl chains, Biochim. Biophys. Acta 1372 (1998) 347–358.

[60] K. Saxena, P. Zimmermann, R.R. Schmidt, G.G. Shipley, Bilayer properties of totally synthetic C16:0-lactosyl-ceramide, Biophys. J. 78 (2000) 306–312.

[61] K. Saxena, R.I. Duclos, P. Zimmermann, R.R. Schmidt, G.G. Shipley, Structure and properties of totally synthetic galacto- and gluco-cerebrosides, J. Lipid Res. 40 (1999) 839–849.

[62] R.D. Koynova, M. Caffrey, Phases and phase transitions of the sphingolipids, Biochim. Biophys. Acta 1255 (1995) 213–236.

[63] T. Rog, I. Vattulainen, A. Bunker, M. Karttunen, Glycolipid membranes through atomistic simulations: effect of glucose and galactose head groups on lipid bilayer properties, J. Phys. Chem., B 111 (2007) 10146–10154.

[64] H.J. Hinz, H. Kuttenreich, R. Meyer, M. Renner, R. Frund, R. Koynova, A.I. Boyanov, B.G. Tenchov, Stereochemistry and size of sugar head groups determine structure and phase behavior of glycolipid membranes: densitometric, calorimetric, and X-ray studies, Biochemistry 30 (1991) 5125–5138.

[65] D.A. Mannock, P.E. Harper, S.M. Gruner, R.N. McElhaney, The physical properties of glycosyl diacylglycerols. Calorimetric, X-ray diffraction and Fourier transform spectroscopic studies of a homologous series of 1,2-di-O-acyl-3-O-(beta-D-galactopyranosyl)-sn-glycerols, Chem. Phys. Lipids 111 (2001) 139–161.

[66] X. Xu, R. Bittman, G. Duportail, D. Heissler, C. Vilcheze, E. London, Effect of the structure of natural sterols and sphingolipids on the formation of ordered sphingolipid/sterol domains (rafts). Comparison of cholesterol to plant, fungal, and disease-associated sterols and comparison of sphingomyelin, cerebrosides, and ceramide, J. Biol. Chem. 276 (2001) 33540–33546.

[67] Z.D. Schultz, I.W. Levin, Lipid microdomain formation: characterization by infrared spectroscopy and ultrasonic velocimetry, Biophys. J. 94 (2008) 3104–3114.

[68] C.D. Blanchette, W.C. Lin, T.V. Ratto, M.L. Longo, Galactosylceramide domain microstructure: impact of cholesterol and nucleation/growth conditions, Biophys. J. 90 (2006) 4466–4478.

[69] W.C. Lin, C.D. Blanchette, M.L. Longo, Fluid-phase chain unsaturation controlling domain microstructure and phase in ternary lipid bilayers containing GalCer and cholesterol, Biophys. J. 92 (2007) 2831–2841.

[70] B.P. van der, M. Lopes-Cardozo, G. van Meer, Sorting of newly synthesized galactosphingolipids to the two surface domains of epithelial cells, J. Cell Biol. 132 (1996) 813–821.

[71] Y.J.E. Björkqvist, T.K.M. Nyholm, J.P. Slotte, B. Ramstedt, Domain formation and stability in complex lipid bilayers as reported by cholestatrienol, Biophys. J. 88 (2005) 4054–4063.

[72] S.M. Alanko, K.K. Halling, S. Maunula, J.P. Slotte, B. Ramstedt, Displacement of sterols from sterol/sphingomyelin domains in fluid bilayer membranes by competing molecules, Biochim. Biophys. Acta 1715 (2005) 111–121.

[73] Megha, E. London, Ceramide selectively displaces cholesterol from ordered lipid domains (rafts): implications for lipid raft structure and function, J. Biol. Chem. 279 (2004) 9997–10004.

[74] I. van Genderen, M.G. van, Differential targeting of glucosylceramide and galactosylceramide analogues after synthesis but not during transcytosis in Madin–Darby canine kidney cells, J. Cell Biol. 131 (1995) 645–654.

[75] S.C. van Ijzendoorn, D. Hoekstra, Polarized sphingolipid transport from the subapical compartment: evidence for distinct sphingolipid domains, Mol. Biol. Cell 10 (1999) 3449–3461.

[76] W.T. Norton, L.A. Autilio, The lipid composition of purified bovine brain myelin, J. Neurochem. 13 (1966) 213–222.

[77] D. Mamelak, M. Mylvaganam, H. Whetstone, E. Hartmann, W. Lennarz, P.B. Wyrick, J. Raulston, H. Han, P. Hoffman, C.A. Lingwood, Hsp70s contain a specific sulfogalactolipid binding site. Differential aglycone influence on sulfogalactosyl ceramide binding by recombinant prokaryotic and eukaryotic hsp70 family members, Biochemistry 40 (2001) 3572–3582.

[78] P. Viani, G. Cervato, S. Marchesini, B. Cestaro, Fluorospectroscopic studies of mixtures of distearoylphosphatidylcholine and sulfatides with defined fatty acid compositions, Chem. Phys. Lipids 39 (1986) 41–51.

[79] F.B. Craves, B. Zalc, L. Leybin, N. Baumann, H.H. Loh, Antibodies to cerebroside sulfate inhibit the effects of morphine and beta-endorphin, Science 207 (1980) 75–76.

[80] H.H. Loh, T.M. Cho, Y.C. Wu, E.L. Way, Stereospecific binding of narcotics to brain cerebrosides, Life Sci. 14 (1974) 2231–2245.

[81] J.R. Yandrasitz, R.M. Cohn, B. Masley, D. DelRowe, Evaluation of the binding of serotonin by isolated CNS acidic lipids, Neurochem. Res. 5 (1980) 465–477.

[82] D. Mamelak, C. Lingwood, The ATPase domain of hsp70 possesses a unique binding specificity for 3′-sulfogalactolipids, J. Biol. Chem 276 (2001) 449–456.

[83] Y. Harada, C. Sato, K. Kitajima, Complex formation of 70-kDa heat shock protein with acidic glycolipids and phospholipids, Biochem. Biophys. Res. Commun. 353 (2007) 655–660.

[84] J.M. Boggs, W. Gao, Y. Hirahara, Signal transduction pathways involved in interaction of galactosylceramide/sulfatide-containing liposomes with cultured oligodendrocytes and requirement for myelin basic protein and glycosphingolipids, J. Neurosci. Res. 86 (2008) 1448–1458.

[85] J.M. Boggs, W. Gao, Y. Hirahara, Myelin glycosphingolipids, galactosylceramide and sulfatide, participate in carbohydrate-carbohydrate interactions between apposed membranes and may form glycosynapses between oligodendrocyte and/or myelin membranes, Biochim. Biophys. Acta 1780 (2008) 445–455.

[86] D.N. Arvanitis, W. Min, Y. Gong, Y.M. Heng, J.M. Boggs, Two types of detergent-insoluble, glycosphingolipid/cholesterol-rich membrane domains from isolated myelin, J. Neurochem. 94 (2005) 1696–1710.

[87] D.A. Brown, J.K. Rose, Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface, Cell 68 (1992) 533–544.

[88] R.J. Stewart, J.M. Boggs, Dependence of the surface expression of the glycolipid cerebroside sulfate on its lipid environment: comparison of sphingomyelin and phosphatidylcholine, Biochemistry 29 (1990) 3644–3653.

[89] W. Weerachatyanukul, I. Probodh, K. Kongmanas, N. Tanphaichitr, L.J. Johnston, Visualizing the localization of sulfoglycolipids in lipid raft domains in model membranes and sperm membrane extracts, Biochim. Biophys. Acta 1768 (2007) 299–310.

[90] M.J. Ruocco, G.G. Shipley, E. Oldfield, Galactocerebroside-phospholipid interactions in bilayer membranes, Biophys. J. 43 (1983) 91–101.

[91] Z. Pernber, K. Richter, J.E. Mansson, H. Nygren, Sulfatide with different fatty acids has unique distributions in cerebellum as imaged by time-of-flight secondary ion mass spectrometry (TOF-SIMS), Biochim. Biophys. Acta 1771 (2007) 202–209.

[92] J.M. Boggs, K.M. Koshy, G. Rangaraj, Influence of structural modifications on the phase behavior of semi-synthetic cerebroside sulfate, Biochim. Biophys. Acta 938 (1988) 361–372.

[93] J.M. Boggs, K.M. Koshy, G. Rangaraj, Interdigitated lipid bilayers of long acyl chain species of cerebroside sulfate. A fatty acid spin label study, Biochim. Biophys. Acta 938 (1988) 373–385.

[94] A. Nabet, J.M. Boggs, M. Pezolet, Study by infrared spectroscopy of the interdigitation of C26:0 cerebroside sulfate into phosphatidylcholine bilayers, Biochemistry 35 (1996) 6674–6683.

[95] K. Saxena, R.I. Duclos, P.K. Zimmermann, G.G. Shipley, Unusual hydration properties of C16: 0 sulfatide bilayer membranes, Biophys. J. 79 (2000) 385–393.

[96] Y.J. Bjorkqvist, S. Nybond, T.K. Nyholm, J.P. Slotte, B. Ramstedt, N-palmitoyl-sulfatide participates in lateral domain formation in complex lipid bilayers, Biochim. Biophys. Acta 1778 (2008) 954–962.

[97] A. Menikh, P.G. Nyholm, J.M. Boggs, Characterization of the interaction of Ca2+ with hydroxy and non-hydroxy fatty acid species of cerebroside sulfate by Fourier transform infrared spectroscopy and molecular modeling, Biochemistry 36 (1997) 3438–3447.

[98] F. Schifferer, H. Beitinger, H. Rahmann, D. Mobius, Effect of calcium and temperature on mixed lipid-valinomycin monolayers. A comparison of glycosphingolipids (ganglioside GT1b, sulphatides) and phosphatidylcholine, FEBS Lett. 233 (1988) 158–162.

[99] S. Tupper, P.T. Wong, N. Tanphaichitr, Binding of Ca2+ to sulfogalactosylceramide and the sequential effects on the lipid dynamics, Biochemistry 31 (1992) 11902–11907.

[100] J.M. Boggs, Lipid intermolecular hydrogen bonding: influence on structural organization and membrane function, Biochim. Biophys. Acta 906 (1987) 353–404.

[101] J.M. Boggs, A. Menikh, G. Rangaraj, Trans interactions between galactosylceramide and cerebroside sulfate across apposed bilayers, Biophys. J. 78 (2000) 874–885.

[102] S. Hakomori, Carbohydrate-to-carbohydrate interaction, through glycosynapse, as a basis of cell recognition and membrane organization, Glycoconj. J. 21 (2004) 125–137.

[103] K.M. Koshy, J. Wang, J.M. Boggs, Divalent cation-mediated interaction between cerebroside sulfate and cerebrosides: an investigation of the effect of structural variations of lipids by electrospray ionization mass spectrometry, Biophys. J. 77 (1999) 306–318.

[104] S. Matuoka, M. Akiyama, H. Yamada, K. Tsuchihashi, S. Gasa, Phase behavior in multilamellar vesicles of DPPC containing ganglioside GM3 with a C18:1 sphingoid base and a 24:0 acyl chain (GM3(18,24)) observed by X-ray diffraction, Chem. Phys. Lipids 123 (2003) 19–29.

[105] P. Rock, M. Allietta, W.W. Young Jr., T.E. Thompson, T.W. Tillack, Ganglioside GM1 and asialo-GM1 at low concentration are preferentially incorporated into the gel phase in two-component, two-phase phosphatidylcholine bilayers, Biochemistry 30 (1991) 19–25.

[106] P. Rock, M. Allietta, W.W. Young Jr., T.E. Thompson, T.W. Tillack, Organization of glycosphingolipids in phosphatidylcholine bilayers: use of antibody molecules and Fab fragments as morphologic markers, Biochemistry 29 (1990) 8484–8490.

[107] T.W. Tillack, M. Allietta, R.E. Moran, W.W. Young Jr., Localization of globoside and Forssman glycolipids on erythrocyte membranes, Biochim. Biophys. Acta 733 (1983) 15–24.

[108] I.E. Mehlhorn, E. Florio, K.R. Barber, C. Lordo, C.W. Grant, Evidence that transbilayer interdigitation of glycosphingolipid long chain fatty acids may be a general phenomenon, Biochim. Biophys. Acta 939 (1988) 151–159.

[109] C.M. Rosetti, B. Maggio, R.G. Oliveira, The self-organization of lipids and proteins of myelin at the membrane interface. Molecular factors underlying the microheterogeneity of domain segregation, Biochim. Biophys. Acta 1778 (2008) 1665–1675.

[110] A. Ferraretto, M. Pitto, P. Palestini, M. Masserini, Lipid domains in the membrane: thermotropic properties of sphingomyelin vesicles containing GM1 ganglioside and cholesterol, Biochemistry 36 (1997) 9232–9236.

[111] C. Reich, M.R. Horton, B. Krause, A.P. Gast, J.O. Radler, B. Nickel, Asymmetric structural features in single supported lipid bilayers containing cholesterol and GM1 resolved with synchrotron X-ray reflectivity, Biophys. J. 95 (2008) 657–668.

[112] S.L. Veatch, S.L. Keller, Separation of liquid phases in giant vesicles of ternary mixtures of phospholipids and cholesterol, Biophys. J. 85 (2003) 3074–3083.

[113] C. Grauby-Heywang, J.M. Turlet, Behavior of GM3 ganglioside in lipid monolayers mimicking rafts or fluid phase in membranes, Chem. Phys. Lipids 139 (2006) 68–76.

[114] C. Grauby-Heywang, J.M. Turlet, Study of the cholesterol-GM3 ganglioside interaction by surface pressure measurements and fluorescence microscopy, Colloids Surf., B Biointerfaces 54 (2007) 211–216.

[115] A.M. Fra, E. Williamson, K. Simons, R.G. Parton, Detergent-insoluble glycolipid microdomains in lymphocytes in the absence of caveolae, J. Biol. Chem. 269 (1994) 30745–30748.

[116] R.G. Parton, Ultrastructural localization of gangliosides; GM1 is concentrated in caveolae, J. Histochem. Cytochem. 42 (1994) 155–166.

[117] C. Gomez-Mouton, J.L. Abad, E. Mira, R.A. Lacalle, E. Gallardo, S. Jimenez-Baranda, I. Illa, A. Bernad, S. Manes, A. Martinez, Segregation of leading-edge and uropod components into specific lipid rafts during T cell polarization, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 9642–9647.

[118] P. Palestini, M. Allietta, S. Sonnino, G. Tettamanti, T.E. Thompson, T.W. Tillack, Gel phase preference of ganglioside GM1 at low concentration in two-component, two-phase phosphatidylcholine bilayers depends upon the ceramide moiety, Biochim. Biophys. Acta 1235 (1995) 221–230.

[119] Y. Ohta, S. Yokoyama, H. Sakai, M. Abe, Membrane properties of binary and ternary systems of ganglioside GM1/dipalmitoylphosphatidylcholine/dioleoyl-phosphatidylcholine, Colloids Surf., B Biointerfaces 34 (2004) 147–153.

[120] C. Yuan, L.J. Johnston, Distribution of ganglioside GM1 in L-alpha-dipalmitoyl-phosphatidylcholine/cholesterol monolayers: a model for lipid rafts, Biophys. J. 79 (2000) 2768–2781.

[121] S.L. Frey, E.Y. Chi, C. Arratia, J. Majewski, K. Kjaer, K.Y. Lee, Condensing and fluidizing effects of ganglioside GM1 on phospholipid films, Biophys. J. 94 (2008) 3047–3064.

[122] R.Y. Patel, P.V. Balaji, Characterization of symmetric and asymmetric lipid bilayers composed of varying concentrations of ganglioside GM1 and DPPC, J. Phys. Chem., B 112 (2008) 3346–3356.

[123] R.Y. Patel, P.V. Balaji, Characterization of the conformational and orientational dynamics of ganglioside GM1 in a dipalmitoylphosphatidylcholine bilayer by molecular dynamics simulations, Biochim. Biophys. Acta 1768 (2007) 1628–1640.

[124] S. Chiantia, J. Ries, G. Chwastek, D. Carrer, Z. Li, R. Bittman, P. Schwille, Role of ceramide in membrane protein organization investigated by combined AFM and FCS, Biochim. Biophys. Acta 1778 (2008) 1356–1364.