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Gangliosides as components of lipid membrane domains

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Cell membrane components are organized as specialized domains involved in membrane-associated events such as cell signaling, cell adhesion, and protein sorting. These membrane domains are enriched in sphingolipids and cholesterol but display a low protein content. Theoretical considerations and experimental data suggest that some properties of gangliosides play an important role in the formation and stabilization of specific cell lipid membrane domains. Gangliosides are glycolipids with strong amphiphilic character and are particularly abundant in the plasma membranes, where they are inserted into the external leaflet with the hydrophobic ceramide moiety and with the oligosaccharide chain protruding into the extracellular medium. The geometry of the monomer inserted into the membrane, largely determined by the very large surface area occupied by the oligosaccharide chain, the ability of the ceramide amide linkage to form a network of hydrogen bonds at the water–lipid interface of cell membranes, the Δ^4 double bond of sphingosine proximal to the water–lipid interface, the capability of the oligosaccharide chain to interact with water, and the absence of double bonds into the double-tailed hydrophobic moiety are the ganglioside features that will be discussed in this review, to show how gangliosides are responsible for the formation of cell lipid membrane domains characterized by a strong positive curvature.

Key words: gangliosides/lipid membrane domains/cell membranes/segregation

Gangliosides

Glycerophospholipids, sphingolipids, and cholesterol are the lipid components of cell membranes. Among these, sphingolipids are minor components. They belong to the external layer of the membrane (Feizi 1985) with the hydrophilic headgroup protruding toward the extracellular environment. Gangliosides, glycosphingolipids that contain sialic acid residues, are components of all animal cell membranes and are particularly abundant in the plasma membranes of neurons.

In 1935, Klenk extracted from the brain of a Niemann–Pick disease patient something of new that he called substance X (Klenk 1935). In the following years, he understood (Klenk 1939) that substance X was a mixture of compounds and he named them "gangliosides". Gangliosides attracted immediately the interest of many investigators, but in spite of this, progresses in elucidating their structures were slow. In 1947, the structure of sphingosine was elucidated (Carter et al. 1947) and in 1955 that of sialic acid (Gottschalk 1955). Finally, in 1963, the first ganglioside structure was described (Kuhn and Wiegandt 1963). Following studies were extensively devoted to fully understand the ganglioside structural complexity, metabolism, cellular topology, biological functions, and pathobiological implications (Macher and Sweeley 1978; Sandhoff and Christomanou 1979; Sandhoff and Conzelmann 1984; Miller-Podraza et al. 1992; Svennerholm et al. 1994). This research is still far to be considered concluded, but today there is a general agreement to consider gangliosides as functional molecules involved in the modulation of enzyme properties and of cell signaling, cell adhesion, and protein sorting (Caputto et al. 1977; Morgan and Seifert 1979; Partington and Daly 1979; Davis and Daly 1980; Leon et al. 1981; Roisen et al. 1981; Rybak et al. 1983; Tsuji et al. 1983; Bremer et al. 1984; Facci et al. 1984; Goldenring et al. 1985; Kim et al. 1986; Kreutter et al. 1987; Chan 1988, 1989; Yates et al. 1989; Bassi et al. 1991; Glebov and Nichols 2004a, b; Lin and Shaw 2005).

Gangliosides are complex lipids with a strong amphiphilic character due to the big saccharidic headgroup and the double-tailed hydrophobic moiety. The lipid moiety of gangliosides, shared with all sphingolipids, is called ceramide (Structure 1) and is constituted by a long-chain amino alcohol, 2-amino-1,3-dihydroxy-octadec-4-ene, whose trivial name is sphingosine (Karlsson 1970), connected to a fatty acid by an amide linkage. Of the four possible configurations of sphingosine, only the 2*S*,3*R* is present in nature (Carter et al. 1947, 1961). The term "sphingosine" is also used to identify structures with shorter and longer alkyl chain and structures with no unsaturation (whose name should be sphinganine).

The oligosaccharide chain of gangliosides is variable because of the sugar structure, content, sequence, and connections. This, together with some variability of the lipid moiety, makes gangliosides a very large family of compounds. Table I shows the main ganglioside structures from the nervous system of several animal species, together with the trivial and correct abbreviations.

Sialic acid is the sugar that differentiates gangliosides from neutral glycosphingolipids and sulfatides. Sialic acid (Schauer 1982) is the name that identifies all the derivatives of 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid or neuraminic acid. Three main sialic acids are known: the 5-*N*-acetyl-, the 5-*N*-acetyl-9-*O*-acetyl-, and the 5-*N*-glycolyl derivatives. Healthy humans have only the first

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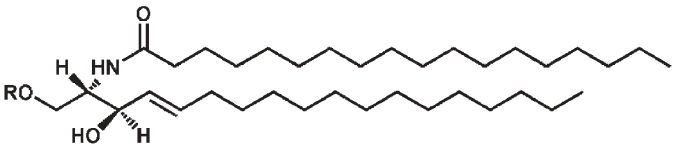
Structure 1



two (Yu and Ledeen 1972; Kamerling and Vliegenthart 1975; Ghidoni et al. 1980; Chigorno et al. 1982). Gangliosides containing polysialyl chains, where the sialic acids are linked together with the ketosidic and ester linkage (ganglioside lactones), have been found in human brains (Riboni et al. 1986).

Gangliosides and lipid membrane domains

The high heterogeneity of the ganglioside oligosaccharide structures should allow the occurrence of specific interactions between proteins and the oligosaccharide chains at the cell membrane surface. Nevertheless, theoretical considerations and experimental data from artificial membranes suggest that the glycolipid physico-chemical properties, such as the lipid transition temperature, the hydrogen-bond network at the lipid–water interface, the geometry of the hydrophilic headgroups, and the carbohydrate–water interactions, can cooperate in governing the membrane domain formation, existence, and organization.

The interest for lipid membrane domains, zones of the membrane with a peculiar composition different from that of the majority of bilayer, became very strong in the last 15 years, when investigations carried out in either artificial or cellular models using a variety of techniques indicated that a small amount of proteins deputed to cell signaling (in general, no more than 0.5–2.5% of the total cell protein content) had a lipid environment highly enriched in sphingolipids and cholesterol. In addition, this information suggested that organization in domains, whose properties are based on their peculiar lipid composition, might be a common feature of biological membranes. Currently, the existence of domains with molecular composition and physical–chemical properties [the best known is probably the inability of some detergents to solubilize membrane lipid domains under certain experimental conditions, given as detergent-resistant membranes (DRM)] distinct from the surrounding membrane environment and the involvement of these domains in regulating cell functions are accepted by many authors. However, most experimental evidence supporting this hypothesis is indirect or, in some aspects, controversial and the actual existence of lipid domains in cellular membranes is still debated (Munro 2003). In addition, in spite of the very high number of articles reporting on lipid membrane domains, now available, the forces that rule their basic organization, stability, and dynamics are only partly understood. As an example of this, an apparent strong interaction between cholesterol and sphingomyelin has been discussed to be the main responsible for the formation of lipid membrane domains. However, it has also been reported (Holopainen et al. 2004) that in some cases, there is no evidence of such a specific interaction (Radhakrishnan et al. 2000; Li et al. 2001, 2003; McConnell and Radhakrishnan 2003).

Several topics related to lipid membrane domains have been recently and extensively reviewed (Barenholz 2004; Chamberlain 2004; Chen et al. 2004; Chini and Parenti 2004; Devaux and Morris 2004; Fielding CJ and Fielding PE 2004; Fullekrug and Simons 2004; Gulbins et al. 2004; Helms and Zurzolo 2004; Harder and Engelhardt 2004; Kahya et al. 2004; Laude and Prior 2004; Leidy et al. 2004; Lommerse et al. 2004; Mukherjee and Maxfield 2004; Nayak and Hui 2004; Parton and Hancock 2004; Resh 2004; Salaun et al. 2004; Sangiorgio et al. 2004; Schuck and Simons 2004; Simons and Vaz 2004; Zuckermann et al. 2004; Barnett-Norris et al. 2005; Hasler and Zouali 2005; He et al. 2005; Hinrichs et al. 2005; Hommelgaard et al. 2005; Hooper 2005; Horejsi 2005; Insel et al. 2005; Ishitsuka et al. 2005; Kenworthy 2005; Lafont and van der Goot 2005; Lagerholm et al. 2005; Langhorst et al. 2005; Li et al. 2005; Lin et al. 2005; Martin et al. 2005; O'Shea 2005; Pietianen et al. 2005; Rajendran and Simons 2005; Rodgers et al. 2005; Rodgers and Smith 2005; van Meer and Vaz 2005; Touyz 2006). Table II shows the lipid contents of DRM fraction prepared from rat cerebellar granule cells in culture. From the table, it remains evident that in spite of the sphingolipid and cholesterol enrichment, glycerophospholipids remain the main components of DRM.

The earliest evidence supporting the existence of lipid domains, conceived as areas in the membrane different in lipid composition from other areas in the membrane, was obtained studying artificial membrane models represented by phospholipid bilayers, containing glycosphingolipids, sphingomyelin, ceramide, and/or cholesterol, by sphingolipid micelles, and by lipid monolayers on an air–water interface or on a solid support.

Membrane lipids, not necessarily sphingolipids (Grant et al. 1974; Knoll et al. 1991; Rock et al. 1991), exist in multiple phases, and this was probably the first evidence leading to the concept of lipid domains. However, in the case of glycosphingolipids, their unique properties, such as the geometry of the monomer inserted into the membrane, the capability of the amide linkage of ceramide to form a network of hydrogen bonds at the water–lipid interface of cell plasma membrane, the Δ^4 double bond of sphingosine near the water–lipid interface, the capability of the oligosaccharide chain to interact with water, and the specific content of saturated alkyl chains, suggest a strong tendency to form segregated compositional domains in phospholipid bilayers. Thus, glycosphingolipids can play an active and primary role in forming and/or stabilizing lipid membrane domains in cells. The evidence supporting this role is discussed in the next three sections of this review.

Starting from the early eighties, this was clearly shown for a number of neutral glycosphingolipids (Tinker et al. 1976; Correa-Freire et al. 1979, 1982; Bunow and Levin 1980; Gambale et al. 1982; Skarjune and Oldfield 1982; Tillack et al. 1982; Barenholz et al. 1983; Thompson et al. 1985; Rock et al. 1990, 1991). Much more controversial appeared the situation for gangliosides, which are unique among glycosphingolipids for their strong amphiphilic character (Sonnino et al. 1994). By means of spin-label probes (Sharom and Grant 1978; Bertoli et al. 1981), gangliosides were shown,

Table I. The main ganglioside structures from the nervous system of mammals

Structure	Series		Abbreviation	Abbreviation
α-Neu5Ac-(2-3)-β-Gal-(1-1)-Cer	Galacto	Gal	GM4	Neu5AcGalCer
α-Neu5Ac-(2-3)-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Lacto	Lac	GM3	II^3Neu5AcLacCer
α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Lacto	Lac	GD3	$II^3(Neu5Ac)_2$LacCer
α-Neu5,9Ac_2-(2-8)-α-Neu5Ac-(2-3)-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Lacto	Lac	*O*-Acetyl-GD3	II^3[Neu5,9Ac_2-(2-8)-Neu5Ac]LacCer
β-GalNAc-(1-4)-[α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-3	Gg_3	GM2	II^3Neu5AcGg_3Cer
β-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-3	Gg_3	GD2	$II^3(Neu5Ac)_2Gg_3$Cer
β-Gal-(1-3)-β-GalNAc-(1-4)- [α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	GM1	II^3Neu5AcGg_4Cer
α-Neu5Ac-(2-3)-β-Gal-(1-3)-β-GalNAc-(1-4)-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	GM1b	IV^3Neu5AcGg_4Cer
α-Fuc-(1-2)-β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	Fuc-GM1	IV^2αFucII^3Neu5AcGg_4Cer
β-GalNAc-(1-4)-β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-5	Gg_5	GalNAc-GM1	II^3Neu5AcGg_5Cer
α-Neu5Ac-(2-3)-β-Gal-(1-4)-β-GlcNAc-(1-3)- β-Gal-(1-4)-β-Glc-(1-1)-Cer	Neolacto-4		3′-LM1	IV^3nLc_4Cer
α-Neu5Ac-(2-3)-β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	GD1a	IV^3Neu5AcII^3Neu5AcGg_4Cer
α-Neu5Ac-(2-3)-β-Gal-(1-3)-[α-Neu5Ac-(2-6)]-β-GalNAc-(1-4)-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	GD1α	IV^3Neu5AcIII^6Neu5AcGg_4Cer
β-GalNAc-(1-4)-[α-Neu5Ac-(2-3)]-β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-5	Gg_5	GalNAc-GD1a	IV^3Neu5AcII^3Neu5AcGg_5Cer
β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	GD1b	$II^3(Neu5Ac)_2Gg_4$Cer
β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5Ac-(2-8,1-9)-α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	GD1b-lactone	II^3[Neu5Ac-(2-8,1-9)-Neu5Ac]Gg_4Cer
α-Fuc-(1-2)-β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	Fuc-GD1b	IV^2αFucII^3Neu5Ac_2Gg_4Cer
α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	GT1a	$IV^3(Neu5Ac)_2II^3$Neu5AcGg_4Cer
α-Neu5Ac-(2-3)-β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	GT1b	IV^3Neu5Ac$II^3(Neu5Ac)_2Gg_4$Cer
α-Neu5Ac-(2-3)-β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5,9A_2c-(2-8)-α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	*O*-Acetyl-GT1b	IV^3Neu5AcII^3[Neu5,9Ac_2-(2-8)-Neu5Ac]Gg_4Cer
α-Neu5Ac-(2-3)-β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	GT1c	$II^3(Neu5Ac)_3Gg_4$Cer
α-Neu5Ac-(2-3)-β-Gal-(1-3)-[α-Neu5Ac-(2-6)]-β-GalNAc-(1-4)-[α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	Chol-1α-a	IV^3Neu5AcIII^6Neu5AcII^3Neu5AcGg_4Cer
β-Gal-(1-3)-[α-Neu5Ac-(2-6)]-β-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	Chol-1β	III^6Neu5Ac$II^3(Neu5Ac)_2Gg_4$Cer
α-Neu5Ac-(2-3)-β-Gal-(1-3)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-6)]-β-GalNAc-(1-4)-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	GT1α	IV^3Neu5Ac$III^6(Neu5Ac)_2Gg_4$Cer
α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-	Ganglio-4	Gg_4	GQ1b	$IV^3(Neu5Ac)_2II^3(Neu5Ac)_2Gg_4$Cer
α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5,9Ac_2-(2-8)-α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-	Ganglio-4	Gg_4	*O*-Acetyl-GQ1b	$IV^3(Neu5Ac)_2II^3(Neu5Ac)_2Gg_4$Cer
α-Neu5Ac-(2-3)-β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	GQ1c	IV^3Neu5Ac$II^3(Neu5Ac)_3Gg_4$Cer
α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-β-Gal-(1-3)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-6)]-β-GalNAc-(1-4)-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	GQ1α	$IV^3(Neu5Ac)_2III^6(Neu5Ac)_2Gg_4$Cer
α-Neu5Ac-(2-3)-β-Gal-(1-3)-[α-Neu5Ac-(2-6)]-β-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	Chol-1α-b	IV^3Neu5AcIII^6Neu5Ac$II^3(Neu5Ac)_2Gg_4$Cer
α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer	Ganglio-4	Gg_4	GP1c	$IV^3(Neu5Ac)_2II^3(Neu5Ac)_3Gg_4$Cer

Table II. Protein and lipid composition in rat cerebellar granule cells differentiated in culture and in DRM fraction prepared by cell lysis with Triton X-100, followed by ultracentrifugation on sucrose gradient Prinetti, Chigorno, Tettamanti, et al. 2000; Prinetti, Chigorno, Prioni, et al. 2001

	Cell homogenate		DRM	
	nmol/10^6 cells	Percent of total lipids	nmol/10^6 cells	Percent of total lipids
Proteins	1.25	3.05	0.02	0.28
Glycerophospholipids	32.84	80.29	3.95	55.39
Cholesterol	4.80	11.73	1.91	26.78
Sphingolipids	2.01		1.25	
Ceramide	0.22	0.53	0.11	1.54
Sphingomyelin	1.00	2.44	0.67	9.39
Gangliosides	0.79	1.93	0.47	6.59
GM3	ND		—	
GM1	0.06		0.04	
GD3	0.04		0.02	
GD1a	0.21		0.11	
GD1b	0.09		0.05	
O-Ac-GT1b	0.08		0.06	
GT1b	0.26		0.17	
O-Ac-GQ1b	0.01		0.02	
GQ1b	0.02		0.67	

even at low concentration, to reduce fluidity and hydrocarbon chain mobility in phosphatidylcholine bilayers, because of lateral cooperative interactions between the ganglioside molecules, i.e., to the formation of ganglioside clusters. This suggested a possible biological relevance of lipid domains (Tettamanti et al. 1980; Bertoli et al. 1981). Membrane fluidity was further decreased by the addition of Ca^{2+}. This suggested the involvement of ganglioside headgroups in the process of ganglioside phase separation (Bertoli et al. 1981). Nevertheless, other studies reported different results, at least for small amounts of gangliosides dispersed in phosphatidylcholine bilayers (Sharom et al. 1977; Bunow MR and Bunow B 1979; Sillerud et al. 1979; Lee et al. 1980; Hinz et al. 1981). Electron microscopy identification of GM1 ganglioside after surface labeling with cholera toxin showed that the lipid was randomly distributed in phospholipid bilayers. On the other hand, with the same technique, asialo-GM1 was found segregated in microdomains (Thompson et al. 1985; Rock et al. 1990). When similar studies were performed using multilamellar liposomes of phospholipid mixtures, which exhibit laterally separated fluid- and gel-phase regions, ganglioside GM1 and its neutral derivative asialo-GM1 were found preferentially into gel-phase regions (Rock et al. 1991). The possible role of glycosphingolipid hydrophobic moiety in determining lipid segregation was also studied (Sonnino et al. 1985). Altogether, results showed (Masserini et al. 2002; Holopainen et al. 2003) that: (1) ganglioside phase separation occurs in phosphatidylcholine bilayers (Goins et al. 1986; Masserini and Freire 1986; Masserini et al. 1988, 1989; Terzaghi et al. 1993; Palestini et al. 1994, 1995; Ferraretto et al. 1997); (2) the extent of ganglioside lateral phase separation depends on the length and unsaturation differences between the ganglioside long-chain base and phosphatidylcholine acyl chains (Masserini and Freire 1986; Masserini et al. 1988, 1989); (3) a decrease in the acyl chain length or an increase in its unsaturation, of ganglioside GM1-induced increased ganglioside distribution in the liquid phase of the bilayer (Palestini et al. 1995); (4) for a given lipid moiety composition, the extent of ganglioside phase separation is dependent on the number of sugars in the oligosaccharide headgroup (Masserini et al. 1988, 1989); (5) the addition of Ca^{2+} promotes the phase separation (Bertoli et al. 1981), by a passive ganglioside exclusion from phosphatidylcholine-rich regions of the bilayer, which are perturbed by Ca^{2+} (Masserini and Freire 1986; Masserini et al. 1989).

When ternary sphingomyelin–GM1–cholesterol vesicles were analyzed by differential scanning colorimetry the formation of separate GM1- and cholesterol-enriched domains was shown (Ferraretto et al. 1997).

Gangliosides in diluted aqueous solution generally form micelles of large molecular mass (Sonnino et al. 1994). This feature allowed us to obtain further information about the lateral segregation of gangliosides using mixed micelle systems, which can be conveniently studied by laser light scattering. In mixed micelles of the two gangliosides GM2 and GT1b, with similar hydrophobic moiety composition, monomers are not randomly distributed in the ellipsoidal micelle (Cantù et al. 1990) (Figure 1). The segregation of one ganglioside with respect to the other in this artificial system is a spontaneous process explained on the basis of the different geometrical properties of ganglioside headgroups. A similar segregation as well due to the geometrical differences between these two gangliosides was shown in mixed micelles of GD1b and GD1b-lactone (Cantù et al. 1991) (Figure 1).

Clusters of globoside in human erythrocytes (Tillack et al. 1983), polysialogangliosides in fish brain neurons (Rahmann et al. 1994), GM3 ganglioside in peripheral human lymphocytes, and Molt-4 lymphoid cells (Sorice et al. 1997) were first visualized by immuno-electron microscopy (Hakomori et al. 1998), but several advanced approaches including single-particle tracking or single fluorophore tracking microscopy (Jacobson et al. 1995; Saxton and Jacobson 1997; Sheets et al. 1997), fluorescence recovery after photobleeching, fluorescence resonance energy transfer (Pralle et al. 2000), and atomic force microscopy are now available (Poole et al. 2004). However, the new approaches gave

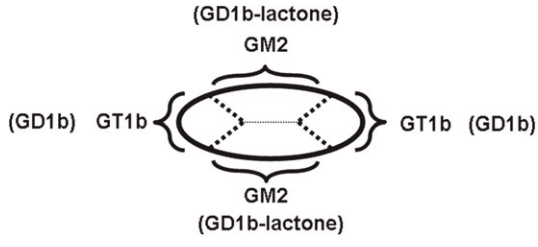


Fig. 1. Schematic representation of the ellipsoidal micelle composed of a mixture of GM2 and GT1b (Cantù et al. 1990) or GD1b and GD1b-lactone (Cantù et al. 1991). In both cases, gangliosides are not randomly distributed on the micelle surface but are segregated, those with the highest surface area being at the edges of the aggregate where the surface has higher curvature.

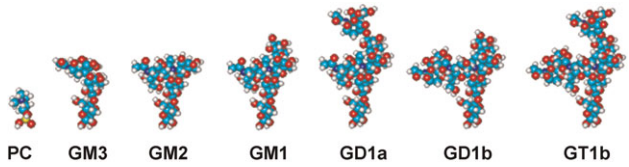


Fig. 2. Schematic representation of the progressive increase in the volume requested by the oligosaccharide chain of gangliosides, due to the progressive increase in its structural complexity. Choline, the biggest phospholipid headgroup, is depicted in the left side of figure for comparison with the glycolipid chains. The hydrophobic moieties are not represented.

results that are sometime conflicting. The most controversial information regards the average size of lipid domains, which ranges from 26 nm to about 2 μm (Sheets et al. 1997; Varma and Mayer 1998; Pralle et al. 2000; Schütz et al. 2000; Poole et al. 2004).

The hydrophilic headgroup of gangliosides

Gangliosides are a very heterogeneous family of compounds with different contents of sugar residues (Table I and Figure 2). Within the ganglioside molecules, all having similar hydrophobic portion and displaying a steric packing of extended and branched different headgroups, the geometrical packing properties can be qualitatively attributed to the hydrophilic moiety (Israelachvili et al. 1976). In fact, different gangliosides inside an aggregate require an interfacial area large enough to provide in the hydrophilic layer a place wide enough to host the oligosaccharide chain and its hydration water (see below). The larger the interfacial area, the smaller the aggregates and the lower the aggregation numbers. Gangliosides with small headgroups, such as GM4 and GM3, form vesicles in aqueous solutions (Sonnino et al. 1990). From GM2 to complex gangliosides of the ganglio-series, the sugar headgroup is so extended that micelles are formed, not vesicles (Sonnino et al. 1994). Moreover, it is clear that increasing the sugar units of the ganglioside leads to a general increase in the molecule interface area, the aggregate becoming more curved and smaller (Table III and Figure 3). Clustering of some components in a membrane system is favored when the components show large differences in the geometrical characteristics of their headgroups, clustering being a spontaneous process due to the minimization of the interfacial free energy. This is the case of gangliosides inserted in a glycerophospholipid surface. The larger is the interfacial area required by ganglioside oligosaccharide structure (Table III), the more positive is the membrane curvature and the more pronounced is the segregation. Figure 2 shows the progressive increase in the volume requested by the oligosaccharide chain with the progressive increase in the complexity. Figure 3 shows the progressive increase in curvature with the progressive increase in the chain complexity. Figure 4 shows an example of the equilibrium between the random distribution and the clustering of a mixture of two gangliosides.

On the other hand, more complex considerations suggest that the contribution to the packing in the aggregate is not merely due to a simple number-of-sugars rule.

The disaccharide -β-Gal-(1−4)-β-Glc-, lactose, is linked to ceramide in all the gangliosides of the Lac and Gg series.

Table III. Hydrodynamic radius R_h (Å), axial ratio R_a/R_b, molecular mass M (kDa) of the aggregates, and number of monomer N and their surface area a_0 (Å^2) in aggregate

		R_h (Å)	R_a/R_b	M (kDa)	N	a_0 (Å^2)
GM4, from bovine brain	Vesicle	≈300		≈18 270	≈18 000	≈80
GM3, from bovine brain	Vesicle	≈250		≈16 700	≈14 000	≈80
GM2, from bovine brain	Micelle	66.0	3.1	630	451	92.0
GM2, from bovine brain, previously warmed at 60°C	Micelle			365	261	96.5
GM1, from bovine brain	Micelle	58.7	2.3	470	301	95.4
GM1, from bovine brain, previously warmed at 60°C	Micelle			320	205	99.5
GM1(d18:1,18:0), previously warmed at 60°C	Micelle	52.8				
GM1(d18:1,18:0), previously warmed at 40°C	Micelle	56.2				
GM1(d18:1,18:0), previously warmed at 25°C	Micelle	58.7				
Fuc-GM1, from pig brain	Micelle	61.0	2.1	394	228	97.8
GD1a, from bovine brain	Micelle	58.0	2.0	418	226	98.1
GD1a, from bovine brain, previously warmed at 60°C	Micelle			318	172	101.7
GalNAc-GD1a, from bovine brain	Micelle	60.0		509	246	97.0
GD1b, from bovine brain	Micelle	52.0	1.8	311	170	100.8
GD1b, from bovine brain, previously warmed at 60°C	Micelle			279	151	104.6
GD1b-lactone, synthesis from bovine brain GD1b	Micelle	57.0	2.1	424	229	97.6
GT1b, from bovine brain	Micelle	53.2	1.8	378	176	100.8
GT1b, from bovine brain, previously warmed at 60°C	Micelle			378	176	100.8

Natural compounds have heterogeneous ceramide moiety. Species prepared by chemical synthesis are indicated with the ceramide structure. Analyses were performed at 30°C (Sonnino et al. 1990, 1994; Cantù et al. 1996; 1999).

Fig. 3. Schematic representation of the ganglioside aggregate curvature related to the size of the ganglioside hydrophilic moieties. From 1–6: GM3, GM1, GM2, GalNAc-GD1a, GD1a, GD1b and GD1a(acetyl). The planar representation of the GM3 bilayer, 1, is only theoretical: to balance the repulsive effect at the bilayer edge, the bilayer needs to close up, forming a vesicle aggregate. The flattest part of the ellipsoidal micelles, 2–6, is represented. For comparison, the quite spherical micellar curvature of the synthetic GD1a containing an acetyl group as acyl chain, 7 (Brocca et al. 1995) is reported. The curvature of the surface aggregates is defined by the amphiphilic character of the monomers (Israelachvili et al. 1976); at constant hydrophobic structure, the features of the ganglioside hydrophilic moieties make the curvature differences. These properties are related to the ganglioside monomers and are expressed in both model membranes and cell membranes.

Many conformers in a very reduced range of minimum energy have been determined on the basis of molecular calculations and a few nuclear magnetic resonance (NMR) data (Acquotti et al. 1990; Siebert et al. 1992). In addition to this, ^{13}C T_1, T_{1r}, and $^{13}C(^1H)$ nuclear Overhauser effect experiments (nOe) (Poppe et al. 1994) suggest that the spatial arrangement of the two linkages can be described by fluctuations in a large energy minimum rather than by the sampling of different conformers with short lifetimes. A character of a low motional freedom is expected for the Glc residue (Skarjune and Oldfield 1982; Jarrell et al. 1987), because of some restrictions of motion imposed by the surrounding membrane surface (Nyholm and Pascher 1993). The addition of sialic acid to lactose gives the oligosaccharide structure of ganglioside GM3. In GM3, the sialic acid is mobile, the ketosidic linkage existing in two main conformations determined by nOe (data on nOe and intra- and interresidual contacts for gangliosides have been extensively reviewed in Acquotti and Sonnino 2000) between galactose and sialic acid that altogether cannot satisfy a single structure (Figure 5). Moving to the more complex gangliosides of the ganglio series 3, 4, and 5, we consider now the trisaccharide sequence -β-GalNAc-(1–4)[α-Neu5Ac-(2–3)-]-β-Gal-, belonging to

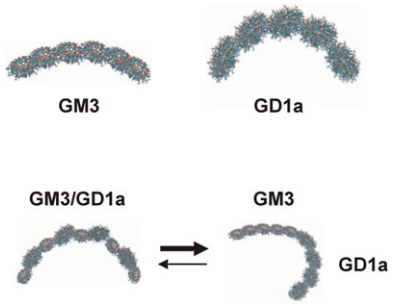


Fig. 4. The upper part of the figure reports the schematic representations of the volume occupied by the GM3 and GD1a oligosaccharides and the deriving aggregate curvatures; the higher the complexity, the higher the curvature. The lower part of the figure shows the schematic representation of a surface containing a mixture of GM3 and GD1a. The strong amphiphilic character of GD1a determines the micellar aggregation of the GM3/GD1a mixture, where the separation of the two components is highly thermodynamically favored.

many compounds. This trisaccharide as a consequence of several interresidual interactions behaves like a rigid block (Figure 5) (Acquotti et al. 1990, 1994; Poppe et al. 1994; Brocca et al. 1996, 1998). The side chain of sialic acid (Figure 6), which is in a rigid conformation (Sabesan et al. 1984; Christian et al. 1987; Poppe et al. 1989; Acquotti et al. 1990), strongly interacts with the *N*-acetylgalactosamine, giving a strong association between the Neu5Ac and GalNAc units, this association being stabilized by a hydrogen bond between the GalNAc amide proton and the Neu5Ac carboxyl group (Brocca et al. 1993). The association between Neu5Ac and GalNAc is decisive for the rigid conformation of the -β-GalNAc-(1–4)[α-Neu5Ac-(2–3)-]-β-Gal-trisaccharide. The shift of GalNAc from the position 4 to position 6 of Gal, as in the synthetic compound 6′-GM2 (Li et al. 1999), changes the trisaccharide dynamics. In the trisaccharide -β-GalNAc-(1–6)[α-Neu5Ac-(2–3)-]-β-Gal-, the GalNAc-(1–6)-β-Gal-glycosidic bond is flexible sampling two main conformations. On the other hand, Neu5Ac, no more interacting with the GalNAc residue, is now more dynamic. Figure 5 shows the conformer representation for the -GalNAc-(1–6)-β-Gal-disaccharide.

From the ganglioseries 3 to the ganglioseries 4, we have the addition of galactose to hexosamine. The disaccharide β-Gal-(1–3)-β-GalNAc- is mobile, allowing the existence of two main conformations of the glycosidic linkage (Figure 5). Thus, GM2, carrying only rigid linkages in the outer portion of the oligosaccharide chain, is present only in one preferred conformation, whereas GM1 carrying the external mobile linkage β-Gal-(1–3)-β-GalNAc is in two and GD1a carrying the external α-Neu5Ac-(2–3)-β-Gal-(1–3)-β-GalNAc in four. According to this, the ganglioside oligosaccharide moieties are represented by a number of conformers which participate to determine the solid angle occupied by the different ganglioside monomers within the surface. It follows that the volume requested to host the GM1 and GD1a oligosaccharide becomes different, in this case much higher, from that expected to host the chain of GM2 plus one or two additional sugar units (Table III). In this regard, it is interesting to analyze ganglioside GalNAc-GD1a. In this ganglioside, the addition of a GalNAc to GD1a gives a second β-GalNAc-(1–4)[α-Neu5Ac-(2–3)-]-β-Gal- rigid trisaccharide directly bound to the first one. Thus, in GalNAc-GD1a, we have two rigid blocks joined together with a mobile linkage, thus reducing the number of conformers from four to two. The four GD1a conformers fill, altogether, ca. 1.760 nm^3, calculated as van der Waals sphere volume, versus ca. 1.420 nm^3 occupied by the two GalNAc-GD1a conformers. Thus, the surface area of GalNAc-GD1a is lower than that of GD1a (Acquotti et al. 1994). Figure 7 shows the van der Waals sphere volumes calculated combining all the minimum energy possible conformers for the GD1a and GalNAc-GD1a oligosaccharides; calculations were performed without taking in account the flexibilities of the Gal–Glc and Glc–Cer linkages.

A further group of gangliosides to be considered is that of structures containing a disialosyl chain linked to the inner galactose. This is the case of GD1b and GT1b, but more complex polysialylated gangliosides have not been studied today. In the tetrasaccharide -β-GalNAc-(1–4)[α-Neu5Ac-(2–8)-α-Neu5Ac-(2–3)-]-β-Gal-, the interresidual contacts between GalNAc and Neu5Ac linked to Gal are not existing,

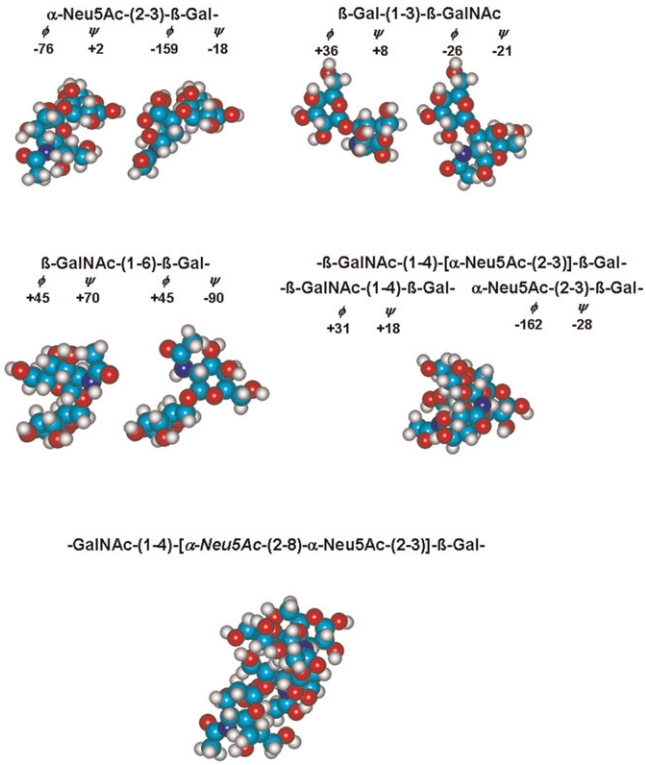


Fig. 5. Glycosidic torsional angle pairs (ϕ, ψ) (accuracy is ±15°) and conformation representation for a series of saccharide linkages common to ganglioseries gangliosides.

but interactions occur between GalNAc and the external Neu5Ac. According to these constraints, the tetrasaccharide chain -β-GalNAc-(1–4)[α-Neu5Ac-(2–8)-α-Neu5Ac-(2–3)-]-β-Gal- is arranged as a rigid structure (Figure 5) having a hole inside, large enough, about 3 Å, to contain cations (Acquotti et al. 1991). Such a conformation confers a bulkier character to the ganglioside portion closer to the hydrophobic–hydrophilic interface and explains the larger value of surface area of GD1b in comparison to that of its isomer GD1a. It is interesting to note that the solid angle required by the disialosyl chain is wide enough to host an additional sialic acid unit in an external position. In fact, although GT1b carries one more sugar linked to the external galactose unit, it requires surface area very similar to that of GD1b.

GD1b, in part, has been found to exist in lactonic form, GD1b-lactone, in human neurons. The conversion process between GD1b and GD1b-lactone has been proposed as a process capable of modulating the activities of membrane proteins (Bassi et al. 1991). When the external sialic acid carboxyl group esterifies the inner sialic acid residue, the interactions between GalNAc and the external Neu5Ac no longer take place, however the rigid conformation of the trisaccharide -β-GalNAc-(1–4)[α-Neu5Ac-(2–3)-]-β-Gal- observed in GM2, GM1, GD1a, and GalNAc-GD1a is restored. This forces a better lining up of the disialosyl chain with the neutral oligosaccharide chain, reducing the angle between the neutral chain and the inner sialic acid axis (Acquotti et al. 1991). Thus, the geometry of GD1b-lactone is closer to GD1a than to GD1b (Table III).

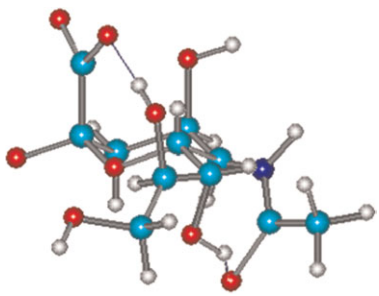


Fig. 6. Conformation of Neu5Ac. The lateral 7–8–9 chain is rigid because of the OH-7 → amide carbonyl and the OH-8 → carboxyl intraresidue hydrogen bonds.

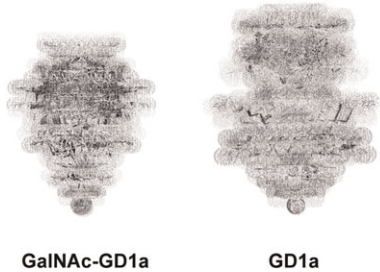


Fig. 7. van der Waals sphere volumes calculated for the GD1a and GalNAc-GD1a oligosaccharide conformers. Dynamics of the Gal–Glc and Glc–Cer linkages was not considered.

The number of carbohydrate rings and the dynamics of glycosidic linkages are the two main parameters determining the large size of the headgroup of gangliosides, but a further factor must be considered. This is the hydrating water that interacts with the oligosaccharide chain. Papers on the topic carbohydrate–carbohydrate interactions are available (Hakomori 2004). Nevertheless, although a good information is available on the head-to-head interactions, data proving direct side-by-side oligosaccharide interactions are very scant. A micellar aggregate of gangliosides (Brocca et al. 1998) where single monomers are very close to each other at the surface should be a good experimental model to study side-by-side oligosaccharide interactions. But, intermonomer carbohydrate–carbohydrate interactions or changes in the oligosaccharide conformation could not be identified by the NMR experiments in homogeneous or mixed micelles of gangliosides. This is due to the large amount of water present in the hydrophilic layer (Ha et al. 1989). In fact, it should be noted that the actual environment of each monomer at the membrane hydrophilic layer includes solvent (Ha et al. 1989). Water is a natural component of the sugar shell being attracted by the hydrophilic character of sugars and by the necessity to avoid repulsion between the negatively charged oligosaccharide (Ha et al. 1989). Calculations performed on GM2 micelles (Cantù et al. 1990) indicated a difference of about 5Å between the dry and hydrated micellar radius. GM2 micelle is an oblate aggregate but calculations can be performed only on an equivalent spherical micelle having the same mass. This does not allow us to know the number but suggests that several molecules of water are interacting with the oligosaccharide chains. This is in agreement with calorimetric studies suggesting that the ganglioside oligosaccharide chains are surrounded by 40–70 water molecules (Bach et al. 1982). A strong interaction between water and GM1 sugars, sialic acid and the inner galactose, was observed by NMR (Brocca et al. 1998). Water bridges between saccharides have been observed in hyaluronan, where they were enough strong to determine and stabilize the three-dimensional structure of the molecule (Heatley and Scott 1988). Of course, these results and considerations would exclude any direct intermonomer side-by-side carbohydrate interactions at the level of cell membrane but are in favor of a specific role of water in organizing a net of hydrogen bonds able to stabilize the glycosphingolipid clustering.

Finally, we recall that the geometry of the ganglioside monomer, determined by the size of the headgroups and their structural differences, has also an important effect in modulating the transition temperature of the lipid moiety, thus modulating the fluidity of the membrane lipid core. The bigger the headgroup, the lower the transition temperature (Table IV). This is a further opportunity to modulate the segregation process as a function of the headgroup structure.

Cellular membranes represent a polymorph system where several lipid organizations can occur and where positive and negative surface curvatures are in sequence. The availability of large hydrophilic headgroup in ganglioside structures showing small differences is a good opportunity to stabilize the membrane lipid domains and subdomains (Vyas et al. 2001) with positive curvature. Figure 8 shows the schematic representation of a caveola where gangliosides are segregated at the edges of the invagination.

Table IV. Transition temperature T_t (°C) of sphingolipids aggregates

	T_t (°C)
GlcCer, from bovine spleen	83.7
LacCer, from bovine adrenal medulla	74.4
Gg_3Cer, by hydrolysis of bovine brain GM2	60.8
Gg_4Cer, by hydrolysis of bovine brain GM1	54.0
GM3, from bovine adrenal medulla	35.3
GM2, from bovine brain	29.3
GM1, from bovine brain	19.5 ± 2
GM1(d18:1,18:0), previously warmed at 60 °C	11.7
GM1(d18:1,18:0), previously warmed at 40 °C	15.0
GM1(d18:1,18:0), previously warmed at 25 °C	17.6
GM1(d20:1,18:0), previously warmed at 60 °C	23.2
Fuc-GM1, from pig brain	13.2
Fuc-GM1(d18:1,18:0), previously warmed at 60 °C	10.0
Fuc-GM1(d20:1,18:0), previously warmed at 60 °C	18.3
GD1a, from bovine brain	15.6 ± 4
GD1a(d18:1,18:0), previously warmed at 60 °C	10.1
GD1a(d20:1,18:0), previously warmed at 60 °C	19.2
GT1b, from bovine brain	7.3
SM, from several sources	35.7 ± 5.8

Natural compounds have heterogeneous ceramide moiety (Maggio et al. 1985; Koynova and Caffrey 1995; Cantù et al. 1999.

The lipid–water interface of gangliosides

The headgroup sizes and the high transition temperature of the hydrophobic chains of gangliosides favor the segregation process; the oligosacharide water environment through intermolecular water bridges stabilizes it. In addition to this, other

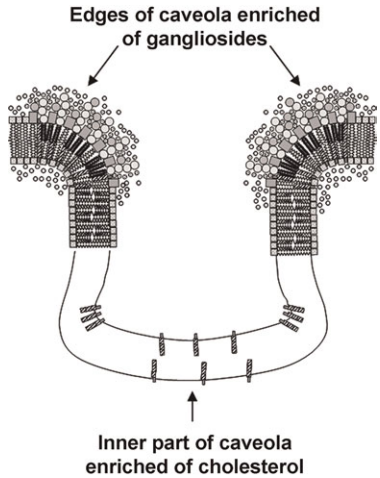


Fig. 8. Schematic representation of a caveolar invagination. The ganglioside segregation at the edges of the caveola should favor the strong positive curvature necessary to link up the negative membrane to the flat membrane environment. Caveolae have been reported to be separated by ganglioside-enriched lipid domains (Iwabuchi, Handa, and Hakomori 1998; Chigorno et al. 2000) and to have a high content of cholesterol that should be mainly in the outer leaflet of the membrane (Schroeder et al. 1991; Igbavboa et al. 1997).

relevant events occur at the membrane surface and participate to stabilize the membrane lipid domains. In fact, the membrane lipid domains exist in cells that contain sphingomyelin but have low amount, or are lacking, of glycosphingolipids (Ostermeyer et al. 1999) and in subdomains with sphingomyelin but a very low content of neutral glycosphingolipids and no content of gangliosides (Iwabuchi, Yamamura, et al. 1998; Chigorno et al. 2000). Thus, some features related to ceramide, the moiety belonging to all sphingolipids, and therefore also to gangliosides, is strictly related to lipid-domain stabilization and there is a general consent that this is related to the portion of ceramide belonging to the water–lipid interface.

At the water–lipid interface, we have the amide group of ceramide as a rigid system comprising six atoms in a planar conformation, together with the hydroxyl group at position 2. The availability of an amide nitrogen, of a carbonyl oxygen, and of a hydroxyl group enables sphingolipids to form hydrogen bonds, acting as hydrogen-bond donors and acceptors at the same time. Therefore, this feature allows sphingolipids to form a stable net of interactions, thus becoming very important in the case of gangliosides whose headgroups per se promote the clustering process. Concerning the other membrane complex lipids, glycerophospholipids do not have this property because they can act only as acceptors of hydrogen bonds and cholesterol has very limited capacity to form hydrogen bonds. The van der Waals forces between hydrocarbon chains have been estimated to about 2–3 kcal per hydrocarbon chain. The formation of hydrogen bonds at the water–lipid interface contributes with 3–10 kcal to the lipid–lipid interaction. Thus, the orientation of the hydrogen-bond donor and acceptor groups of sphingolipids optimal to form lateral interactions and the considerable increase in stability in the lipid association are very good candidates to promote the formation of a membrane rigid zone where a network of hydrogen-bond-connected lipids are segregated together with cholesterol.

The hydrophobic chains of gangliosides

The group has a perpendicular orientation toward the axes of the two hydrocarbon chains, whose parallel orientation is stabilized by the Δ^{4-5} unsaturation of sphingosine (Pascher 1976). Thus, the ceramide moiety can be considered a rigid structure and addition of glycosphingolipids to cells was shown to reduce the original membrane fluidity (Bertoli et al. 1981).

Membrane complex lipids are highly heterogeneous in their lipid moieties. Many of them contain unsaturated alkyl chains. This is an essential requirement to have fluid membranes so that protein conformational changes and lipid organization changes are allowed. But, complex lipids with saturated chains are also membrane components. In the membrane, these components that contain rigid saturated alkyl chains with high transition temperatures are excluded from those that contain unsaturated chains with low transition temperature. Phosphatidylcholine is the major membrane glycerophospholipid. It comprises for several molecular species, differing in the lipid moiety. Within these, dipalmitoylphosphatidylcholine is the main species in DRM (Prinetti et al. 2001; Pitto et al. 2002). Palmitic and stearic acids are the main fatty acids of gangliosides. Thus, if we recall that over 60% of total membrane gangliosides are inside the lipid-domain fractions, it follows that lipid domains are highly enriched of unsaturated chains (Maggio et al. 1985; Pitto et al. 2002). In this rigid environment, cholesterol, which alone has a melting point of 148.5 °C, would find a correct position.

Conclusions

Gangliosides are the components of the membranes of all living organism cells and are particularly abundant in the plasma membranes of neuronal cells. They show a strong amphiphilic character, being constituted by a two-tail hydrophobic moiety, ceramide, and a structurally very variable hydrophilic headgroup. In considering the forces that drive the formation of membrane areas with a selected lipid composition leading to the creation of a liquid-ordered phase environment, many authors emphasized the role of cholesterol and of specific lateral interactions with other membrane lipids (i.e., sphingomyelin) and proteins bearing cholesterol-interacting motifs. However, as discussed in the present review, theoretical considerations and experimental data suggest that gangliosides, by means of the size and peculiar features of their headgroups, of the possibility to generate hydrogen bonds at the water–lipid interface, and of the high content of saturated hydrophobic tails, play an active role in the organization and maintenance of membrane lipid domains, zones of the membrane with reduced fluidity. Cholesterol and saturated chain glycerophospholipids tend to partition in these membrane areas, contributing to their physico-chemical and biological properties. Moreover, the segregation of gangliosides within the restricted membrane areas together with several areas where proteins involved in the processes of cell signaling poses the molecular basis for lateral interactions between ganliosides and membrane proteins, providing new clues to understand the modulation of membrane protein activity that underlies many biological roles of ganglioside themselves. In conclusion, gangliosides play an active rather than passive role in the formation, stabilization, dynamics in the space and time, and biological functions of lipid membrane domains.

Conflict of interest statement

None declared.

Abbreviations

DRM, detergent-resistant membranes; NMR, nuclear magnetic resonance; nOe, nuclear Overhauser effect.

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