Minireview

Membrane properties of sphingomyelins

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Abstract Sphingomyelin and phosphatidylcholine are important components in the external leaflet of cellular plasma membranes. In this review we compare the structure of these lipid molecules, with emphasis on the differences in hydrogen bonding capacity and membrane properties that arise from the small but significant differences in molecular structure. The membrane properties of sphingomyelins and the implications that these have, or might have, in biological membranes and for raft function are further discussed.

Key words: Cholesterol; Lipid interaction; Raft property; Lipid miscibility; Lateral heterogeneity

1. Introduction

In the 1880s a lipid compound was isolated from brain tissue by Johann L.W. Thudicum, who gave it the name sphingomyelin (SM) [1]. The structure of this sphingophospholipid was reported in 1927 to be *N*-acyl-sphingosine-1-phosphorylcholine [2]. Today we know that SM is an important component of the plasma membranes of eukaryotic cells [3]. In most mammalian tissues the SM content ranges from 2 to 15% of the total organ phospholipid depending on the tissue studied [3]. Even higher levels of SM are found in, for example, erythrocytes, ocular lenses, peripheral nerve tissue and brain [3,4]. SM functions as a structural component in biological membranes together with other phospholipids, glycolipids, cholesterol and some integral membrane proteins. In addition to its structural role SM also participates in cell signalling. Products of SM metabolism, like ceramide, sphingosine, sphingosine-1-phosphate and diacylglycerol, are important cellular effectors and give SM a role in cellular functions like apoptosis, ageing and development (for reviews see for example [5–7]). The importance of SM, both structurally and functionally, has kept an ongoing interest in this membrane lipid in recent years (see for example [8]).

2. Molecular structure of natural sphingomyelins

The structures of SM and phosphatidylcholine (PC) are presented in Fig. 1. Both lipids have phosphorylcholine as the polar head group. The structures of SM and PC differ significantly in the interfacial and hydrophobic parts of the molecules. The most common base in mammalian SM is sphingosine (1,3-dihydroxy-2-amino-4-octadecene), with a *trans*-double bond between C_4 and C_5 [9,10]. The saturated dihydro derivative, called sphinganine (1,3-dihydroxy-2-amino-octadecane) is also common, although present in much smaller amounts than sphingosine [11]. Human lens membranes are an exception, containing high levels of dihydrosphingomyelin [12]. Smaller amounts of other bases varying in length, degree of hydroxylation and branching can also be found in nature [9]. The enantiomeric configuration of the sphingoid base in natural sphingomyelins is always D-*erythro* (2S,3R) [13].

Natural SMs usually constitute a mixed population with the amide-linked acyl chain differing widely in length (from 16 to 24 carbons) [11]. The SM acyl chain composition varies between tissues, although a common feature is that the chains are unusually long, giving the molecules an asymmetric nature. Most tissues contain sphingomyelins with 16:0, 18:0, 22:0, 24:0 and $24{:}1^{cis\Delta15}$ acyl chains [14–16]. PCs usually have moderately long (16–18 carbons) acyl chains of approximately equal length, with the *sn*-1 chain being saturated and the *sn*-2 chain unsaturated with one or more *cis*-double bonds (about 1.1–1.5 double bonds per molecule) [10]. The position and number of such double bonds largely affect the interaction with other membrane components, such as sterols or proteins. In SM there is a high frequency of saturated amide-linked acyl chains with an average of only 0.1–0.35 *cis*-double bonds per molecule [11]. When present, the *cis*-double bond in natural SMs is usually located far away from the interface, as in nervonic acid ($24{:}1^{cis\Delta15}$) with a double bond at C_{15}.

The interfacial regions of SM and PC also differ significantly from each other. SM has the amide group, a free hydroxyl on C_3 and the *trans*-double bond between C_4 and C_5 in this region [10]. The amide and hydroxyl groups can act as hydrogen bond donors and acceptors, whereas the amide carbonyl in SM can only act as hydrogen acceptor [17]. PC with two ester carbonyls in this region has only hydrogen bond-accepting features. These interfacial differences give SMs the unique ability to form both intra- and intermolecular hydrogen bonding. Nuclear magnetic resonance (NMR) spectroscopic studies have recently revealed that SMs form a fairly strong intramolecular hydrogen bond between the sphingosine hydroxyl and the phosphate ester oxygen in both monomeric and aggregated states [4]. Such a hydrogen bond, unique for the naturally occurring D-*erythro*-diastereomer of SM, was

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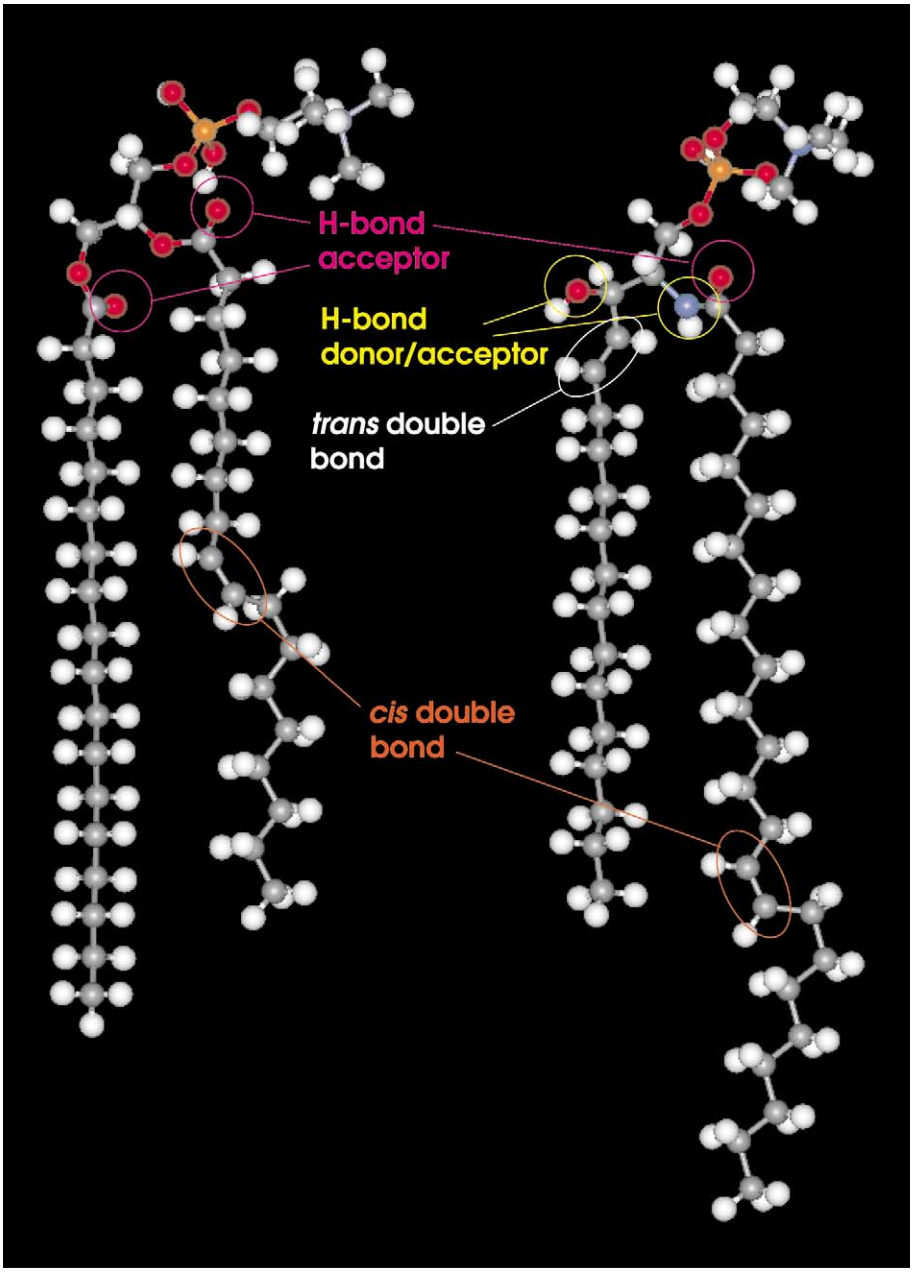

Fig. 1. A comparison of the molecular structure of a representative phosphatidylcholine (1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, left in figure) and a sphingomyelin (*N*-nervonyl-sphingomyelin, right in figure). The highlighted differences in structure and hydrogen-bonding properties of the two molecules are discussed in the text.

also suggested earlier on the basis of NMR [18]. The *trans*-double bond seems to affect the strength of the hydrogen bonding interactions, leading to weaker intramolecular, but stronger intermolecular hydrogen bonds in dihydrosphingomyelin assemblies compared to SM [4,19].

3. Properties of SMs in membranes

SM and PC have fairly cylindrical molecular shapes, making them form bilayers when hydrated to minimise free energies [20]. The polar head groups of the choline phospholipids take a similar orientation, roughly parallel to the surface in the lipid bilayer. The conformations of the first few carbons of the amide-linked and *sn*-2 chains are also very similar. On average, the acyl chains are initially aligned orthogonal to the sphingoid base or *sn*-1 chain, but bend sharply between C_2 and C_3, taking a roughly parallel alignment with respect to the sphingoid base or *sn*-1 chain, respectively [21–23].

Compared to the studies done with PCs, relatively few studies on the physical properties of SMs have been reported. SMs exhibit a more complex membrane behaviour than PCs. The T_m of most natural SMs is close to physiological temperature, which is high compared to that of natural PCs [11,24–26]. However, the thermotropic behaviour of synthetic acyl chain-defined SMs resembles that of corresponding synthetic PCs. SMs with saturated acyl chains ranging from 16 to 24 carbons in length have their main transition somewhere between 37 and 48°C, with an increase, although not linear, in melting temperature with acyl chain length [24]. The T_m for pure D-*erythro*-*N*-16:0-SM has been shown to be about 41°C, with a small pre-transition at 29°C [26,27]. This behaviour resembles that of di-16:0-PC [10,25]. For pure D-*erythro*-*N*-18:0-SM a single chain melting transition has been observed at about 45°C [28]. Bruzik, however, was able to find four distinct gel phases in D-*erythro*-*N*-18:0-SM samples studied by ^{31}P-NMR, indicating complex phase behaviour for these lipids [29].

SMs with larger mismatch in length between the *N*-acyl chain and the sphingosine backbone exhibited more complex thermotropic behaviour with multiple transitions when studied by DSC [30]. The same phenomenon has been observed with corresponding asymmetric PCs and attributed to chain interdigitation in the bilayer (reviewed in [25]). The electron-density profiles of long-chain (20:0, 22:0, 24:0) SMs were also found to suggest chain interdigitation [31]. When reviewing the literature on the thermotropic behaviour of SM, Koynova and Caffrey saw that the transition enthalpy exhibited a discontinuity and the increase in T_m with acyl chain length levelled out at an acyl chain length of 20 carbons [24]. They suggested that a change in chain packing from non-interdigitated to interdigitated occurs at this acyl chain length [24]. As an example, the thermogram for D-*erythro*-*N*-24:0-SM showed two maxima at 39.3 ± 0.05°C and 45.9 ± 0.05°C, which were assigned to the presence of two different gel phases with different degree of hydrocarbon interdigitation [26]. Interdigitation has been suggested to lead to non-ideal mixing with other membrane lipids, as evidenced by the non-ideal mixing of di-14:0-PC with D-*erythro*-*N*-24:0-SM compared to a nearly ideal mixing with D-*erythro*-*N*-16:0-SM [26]. Since chain mismatch exists in natural SMs, the mixing of such SMs with other, mostly unsaturated and nearly symmetric lipids in the plasma membranes of cells can also be highly non-ideal, maybe leading to the formation of lateral domains [10].

Due to the *cis*-double bond at C_{15} the naturally abundant species D-*erythro*-*N*-24:1$^{\Delta 15}$-sphingomyelin has a T_m similar to *N*-14:0-sphingomyelin, somewhere in the region between 25 and 28°C [30,32,33]. The high T_m values for SM are in general less affected by the insertion of *cis*-unsaturations into the acyl chains than other phospholipids [24]. This phenomenon has been attributed to stabilisation of the bilayers by hydrogen bonding [24]. The amide group in SM has recently been indicated in intermolecular hydrogen bonding and water bridging based on NMR spectroscopic studies [4]. An X-ray study of SMs in monolayers also provided new evidence of hydrogen bonding in the head group and interfacial region [34].

Also, the hydration behaviour of natural SMs differs from that of PCs, although these two lipids have the same head group [35]. A recent study with natural SMs from different sources showed complex hydration behaviour for SM, when compared to PC [36]. For PC the number of water molecules in the hydration shell of the polar head group increases when the bilayer goes from gel to liquid-crystalline phase, with the water molecules becoming even more ordered when the disorder in the acyl chains increases [37]. For SMs the behaviour varied widely between SMs derived from different sources. No molecular explanation for the difference in hydration between the choline phospholipids has yet been presented, but it was speculated to depend on a stronger interaction, most likely hydrogen bonding, between the hydrocarbon chain and the head group in SM [36]. Such intramolecular interactions are largely affected by the interfacial part of the SM molecule, such as the stereochemistry and the presence of the *trans*-double bond of the sphingoid base [4,18,19].

4. Cholesterol and SM interact in biological and model membranes

In the plasma membranes of cultured cells the tendency of SM and cholesterol to interact with one another has been studied and there is strong evidence to suggest a preferential interaction between these two lipids. They co-localise in the plasma membranes and at the surface of low-density lipoprotein particles [38,39]. The regulation of cholesterol and SM content in the plasma membranes of cells is also coordinated (recently reviewed in [38,40]). The interactions between cholesterol and different phospholipid classes was recently reviewed by Ohvo-Rekilä and co-workers [41].

Cholesterol affects the physical properties of the surrounding phospholipids when it is incorporated into membranes [42]. Changes in enthalpy and cooperativity of the transition from gel to liquid-crystalline phase occur in the presence of cholesterol and at high cholesterol concentrations the transition is eliminated and the only phase present is a liquid-ordered phase [42,43]. The liquid-ordered phase is in many biophysical respects an intermediate between the gel and liquid-crystalline phases in the bilayer, with lateral motional rates similar to those in the fluid bilayer although the acyl chain order and phospholipid area compressibility values are more as in the gel-state phospholipids [42,44].

The interaction with cholesterol is very sensitive to the length and degree of unsaturation of the acyl chains of the phospholipid [45,46]. Cholesterol–PC interactions are known to depend on how closely the hydrophobic lengths of the sterol and the PC molecules in the bilayer are matched with each other [47,48]. Cholesterol desorption studies indicate that cholesterol interacts favourably with SMs even when their acyl chains are long [49]. Spontaneous cholesterol transfer is known to be dependent on the packing density in the donor membrane [45]. A decrease in packing density by, for example, unsaturations of the acyl chains of the phospholipids or higher curvature of the membrane will consequently facilitate cholesterol desorption [45,50]. Greater lateral packing density in SM-containing membranes has been proposed as the reason for lowering the rate of spontaneous cholesterol transfer from SM-containing membranes [51]. The molecular packing in membranes of cholesterol and SM is also known to differ from that of cholesterol/PC membranes [33,52,53]. The compressibility of SM/cholesterol membranes was actually much lower than that of PC bilayers, with equal cholesterol content, as evidenced by the micropipette aspiration technique and monolayer compressibility studies [33,53]. Also, water permeability was lower in cholesterol/SM than in cholesterol/PC bilayers, indicating tighter packing [53]. This difference in molecular packing may partly be explained by greater van der Waals attractive forces between the saturated acyl chains of SM and the rigid cholesterol ring backbone [35].

During the last decade atomic level computer simulations of improving quality have been performed on mixed membranes with phospholipids and cholesterol. These studies suggest that the cholesterol hydroxyl group may be hydrogen-bonded to the PC carbonyl and phosphate oxygens [54–56]. In the case of sphingolipids and anionic lipids, hydrogen bonding to their polar head groups and interfacial regions seems to be even more likely, especially since cholesterol has been shown to interact differently with different phospholipid classes [41,42]. Current knowledge suggests that the interaction between cholesterol and SM is stabilised by hydrogen bonding [33,57–59]. This hydrogen bonding does not seem to involve the 3-hydroxyl group of SM, since replacement of this group hardly affected the interaction with cholesterol in either vesicles or monolayers [51,60]. Replacement of the amide group linking the acyl chain to the sphingoid backbone in SM with an ester-linkage, on the other hand, weakened the interaction with cholesterol [58]. New evidence for intermolecular hydrogen bonding between the amide group in SM and the 3-hydroxyl of cholesterol came recently from an IR spectroscopic study that showed a large effect of cholesterol on the amide frequency [59].

5. SM and the lateral heterogeneity in membranes

Lipids and proteins are highly organised in biological membranes. The different lipid species are asymmetrically distributed over the outer and inner membrane leaflets. SM and PC are major constituents of the external leaflet of the plasma membranes of cells. Results obtained during the last decade show a substantial lateral organisation of both lipids and proteins in biological membranes as well [23,61–63]. Sphingolipids, including SM, together with the neutral lipid cholesterol, have been implicated in lateral domain or 'raft' formation in biological membranes. These domains have been suggested to take part in cellular processes, such as signal transduction, membrane trafficking and protein sorting (for excellent recent reviews on this subject see [63,64]).

The formation of lateral 'rafts' in biological membranes is supposed to be driven by lipid–lipid interactions, which are largely dependent on the structure and biophysical properties of the lipid components, and result in detergent-resistant domains [61,63]. Model membrane studies have an important role in this field since many questions have been left open by cell-biological studies. Formation of domains in mixed lipid bilayers is favoured by the presence of long-chain saturated sphingo- and phospholipids as well as by physiological proportions of cholesterol [23,65]. It has long been known that glycosphingolipids tend to form microdomains in a fluid, phospholipid-based bilayer. Glycosphingolipids are highly saturated in nature and have high T_m as well as the opportunity to form an extensive hydrogen-bonding network in the head group region and they can therefore form detergent-insoluble gel-phase domains [23,66,67]. Because of their structure glycosphingolipids could be regarded as good candidates for interacting with cholesterol. However, cholesterol has only moderate effects on domain formation by at least some of these glycosphingolipids [68] and in the presence of both SM and glycosphingolipids cholesterol prefers to interact with SM [67].

The rafts co-existing with the fluid matrix of the plasma membrane have been proposed to be in the liquid-ordered phase [66,69]. Cholesterol, although not essential for sphingolipid domain formation, seems to be essential for the formation of the liquid-ordered phase. Fluorescence quenching studies have also shown that the amount of liquid-ordered phase correlates with the detergent-insoluble fraction of cholesterol-containing model membranes [66,70]. Cholesterol promotes phase separation of saturated SMs [71] and SM needs cholesterol to be detergent-insoluble [66,72]. In a ternary system of natural SM, diunsaturated PC and cholesterol, lateral liquid-ordered phase domains of SM and cholesterol were observed at cholesterol concentrations over 30 mol% [73]. In recent studies cholesterol and SM formed microscopically observable circular, liquid-ordered phase domains in supported bilayers and giant unilamellar vesicles [74,75]. These domains could be observed only below the T_m of the SM species used and saturated acyl chains were needed for the formation [74,75]. The formation of a liquid-ordered phase is highly dependent on acyl chain structure of the phospholipids and cholesterol can also interact with saturated PCs forming liquid-ordered domains [70,75–78]. The exact phase behaviour of cholesterol/phospholipid mixtures is very much dependent on the molecular structure of the phospholipid present and immiscible liquid-ordered phases with different degrees of order may form with different phospholipids, such as saturated PCs and SMs [79]. However, cholesterol was not able to form lateral domains with saturated phosphatidylserines or phosphatidylethanolamines, which indicates the importance of the phospholipid head group [75].

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