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Miscibility of acyl-chain defined phosphatidylcholines with *N*-palmitoyl sphingomyelin in bilayer membranes

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Abstract

In this study we have used differential scanning calorimetry (DSC) to study the miscibility of different saturated phosphatidylcholines (PCs) with D-*erythro*-*N*-palmitoyl-sphingomyelin (16:0-SM). Information about the miscibility was obtained by observing the thermotropic phase behavior of binary mixtures of saturated PCs and 16:0-SM. The results obtained showed that PC miscibility in 16:0-SM was markedly affected by the PC acyl-chain composition. According to phase diagrams prepared from DSC data and the mid-transition temperatures of the main phase transition, the PC which formed the most ideal mixture with 16:0-SM was di-14:0-PC. However, the cooperativity of the main transition in PC/16:0-SM bilayers increased until the average acyl-chain length in the PC reached 15 carbons. Based on the criteria of the most ideal miscibility or the highest cooperativity of the main transition, we conclude that di-14:0-PC, 15:0/15:0-PC, and 14:0/16:0-PC interacted most favorably with 16:0-SM in bilayer membranes. Di-16:0-PC, to which 16:0-SM is often compared in biophysical studies, showed much less ideal miscibility.

Keywords: Phospholipid; Phase transition; Model membrane; Cooperativity; Differential scanning calorimetry

1. Introduction

Sphingomyelin and phosphatidylcholine are common components in mammalian membranes. Together they make up over 50% of the total membrane phospholipids in mammalian membranes [1]. In most tissues the SM concentration is about 2–15% of the total phospholipid concentration [2]. The cellular SM is enriched in the plasma membrane, where it is believed to form functional micro domains or lipid rafts together with cholesterol and other sphingolipids [3,4]. These functional domains are thought to be involved in protein sorting, membrane trafficking and cellular signaling events [5].

Both PC and SM are phospholipids with a phosphocholine head group. The significant differences between SM and PC arise from the fact that they are synthesized from different precursors. PC has a glycerol backbone to which two acyl-chains are linked through ester bonds in *sn*-1 and *sn*-2 position, while SM has a sphingoid backbone with an amide-liked acyl-chain. These structural differences give SM and PC rather different membrane properties [1,6,7]. The potential for hydrogen bonding is different for SM and PC. Since SM has both hydrogen bond donating and accepting groups, while PC has only hydrogen bond accepting groups, it is expected that SM forms more complicated hydrogen bonding networks than PC [8].

Although the membrane properties of both SM and PC are quite well characterized, there is only limited information available on the interactions between SM and PC. Natural PC and SM, which differ markedly in acyl-chain

Abbreviations: 16:0-SM, D-*erythro*-*N*-palmitoyl-sphingomyelin; SM, sphingomyelin; PC, phosphatidylcholine; DSC, differential scanning calorimetry; T_m, mid temperature of the gel to liquid-crystalline phase transition; $T_{1/2}$, half-width of the gel to liquid-crystalline phase transition; X_{SM}, the molar fraction of sphingomyelin

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length and degree of unsaturation, have been shown to phase-separate in a temperature- and composition-dependent manner [9]. Saturated PC and SM with similar acyl-chain lengths have, on the other hand, been shown to be completely miscible in a broad temperature interval [10–12], but it is also clear from these studies that the miscibility between SM and PC is dependent on the length of the acyl-chains. To our knowledge, there are no systematic studies on how the length of the acyl-chains in SM and PC affects the way these lipids mix and interact in bilayer membranes. The membrane properties of SM and PC have been compared in several studies, and it has been shown that the interactions between these phospholipids and cholesterol are acyl-chain-dependent [13,14].

In this study, we used differential scanning calorimetry (DSC) to investigate the miscibility of D-*erythro*-*N*-palmitoyl-sphingomyelin (16:0-SM) and a series of saturated PC molecules. From the results, we obtained information about how favorably the different PC molecules mixed with 16:0-SM.

2. Experimental procedures

2.1. Material

D-*erythro*-*N*-palmitoyl-sphingomyelin (16:0-SM) was purified from egg yolk sphingomyelin (Avanti Polar Lipids, Alabaster, AL, USA) by reverse-phase HPLC (SP 250/10 Nucleosil 100-5-C18) using 5 vol.% water in methanol as eluent (at 1 ml/min, column temperature 40 °C). The fatty acid and long-chain base composition of the product was verified by mass spectroscopy (Micromass Quattro II, Manchester, UK). All symmetrical phosphatidylcholines, 1-myristoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine, 1-myristoyl-2-stearoyl-*sn*-glycero-3-phosphocholine, and 1-myristoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (lyso-PC), were purchased from Avanti Polar Lipids. Fatty acids were obtained from Larodan Fine Chemicals (Malmö, Sweden). Asymmetrical phosphatidylcholines were synthesized from lyso-PC and free fatty acids as previously described [14]. In brief, lyso-PC, fatty acid, dicyclohexylcarbodiimide and dimethylaminopyridine (both from Fluka, Buchs, Switzerland) were dissolved in dry chloroform containing Molecular Sieves 4A. The sample was incubated at room temperature for 6 h, after which the phospholipids were initially purified according to Kaluzny et al. [15]. Pure PC was obtained by reverse phase HPLC (SP 250/10 Nucleosil 100-5-C18). The sample was eluted with 100% methanol at a rate of 1 ml/min. Stock solutions of lipids were prepared in hexane/2-propanol (3:2, v/v), stored in the dark at −20 °C, and warmed to ambient temperature before use. The water used was purified by reverse osmosis followed by passage through a Millipore UF Plus water purification system, to yield a product with a resistivity of 18.2 MΩ cm.

2.2. DSC

DSC measurements were performed with a Calorimetry Sciences Nano II differential scanning calorimeter (Provo, UT, USA). The samples were evaporated under a constant flow of N_2, after which they were placed under vacuum for 1 h. The dry lipids were hydrated in 60 °C water and sonicated for 1 min at 60 °C in a Bransonic 2510 bath sonicator (Branson Ultrasonics, CT, USA), resulting mostly in large multilamellar vesicles. The final concentration of phospholipid in the solutions was 1.4 mM, and the molar content of 16:0-SM in the samples was 0, 25, 50, 75 or 100 mol%. The suspensions were degassed under vacuum before being loaded into the DSC. Heating scans were run at a rate of 0.5 °C/min. Data analysis was performed using software provided by the DSC manufacturer (CpCalc 2.1) and Microcal Origin 6.0.

The ideality of mixing in the binary phospholipid mixtures was assessed according to Mabrey and Sturtevant [16]. The ideal phase diagram was calculated according to

$$X_B^{(l)} = (1-\alpha)/(\beta-\alpha); X_B^{(s)} = \beta X_B^{(l)} \quad (1)$$

where the subscripts refer to the liquidus (l) and the solidus (s) curve, and the quantities α and β are defined as

$$\alpha = \exp\left[\frac{\Delta H_A}{R}\left(\frac{1}{T} - \frac{1}{T_A}\right)\right];\ \beta = \exp\left[\frac{\Delta H_B}{R}\left(\frac{1}{T} - \frac{1}{T_B}\right)\right] \quad (2)$$

where ΔH_A and ΔH_B are the transition enthalpies of the pure lipids and T_A and T_B are their absolute transition temperatures. The onset and completion temperatures of the measured transition data were corrected for the contributions to the total transition widths of the pure phospholipids, as described in Ref. [16].

3. Results

3.1. Miscibility of saturated symmetric diacyl-PC molecules and 16:0-SM

Using DSC, we studied the thermotropic behavior of phospholipid bilayers composed of 16:0-SM and synthetic symmetric diacyl-PCs in which the acyl-chain length was varied between 12 and 16 carbons. By observing the gel to liquid-crystalline phase transition in this series of binary mixtures, we obtained information about the chain length dependence of the interactions between PC and SM. Fig. 1 shows the T_m of the main transition for all binary mixtures that were used (except for the di-12:0-PC/16:0-SM, in which the heat capacity function contained separate transition peaks, indicating that the two lipids segregated into separate domains (data not shown)). The T_m for the phospholipid mixtures increased almost linearly with both

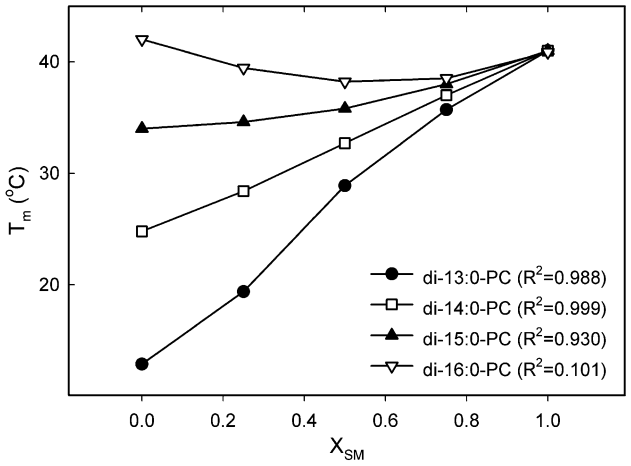


Fig. 1. The main phase transition temperatures of binary mixtures of diacyl-PC and 16:0-SM. Phospholipid membranes with varying composition were heated at a rate of 0.5 °C/min. The linearity of the T_m functions was evaluated by performing a linear fit on the data. The R-squared values from these fits are shown in the graph.

di-13:0-PC and di-14:0-PC content (Fig. 1). With mixtures containing PC molecules with longer acyl-chains (15 and 16 carbons), the T_m increased less linearly, possibly indicating that these symmetric PC molecules interacted less favorably with 16:0-SM. No clear correlation between transition enthalpy and PC acyl chain length was seen in any of the tested binary mixtures.

From the thermograms shown in Fig. 2, it is clear that the cooperativity of the gel to liquid-crystalline phase transition was also affected by the acyl-chain composition in the PC molecules. Some evidence for phase separation was also seen in binary mixtures of di-13:0-PC and 16:0-SM. In Fig. 3 the cooperativity of the phase transition is shown as the half widths ($T_{1/2}$) of the transitions. Fig. 3 shows $T_{1/2}$ for different molar fractions of SM as a function of the PC acyl-chain length. As clearly shown in the figure, the half-width of the transitions of the SM-PC mixtures became smaller as the acyl-chain length was increased, although the half-widths of the pure PCs did not change markedly with acyl-chain. This observation indicates that the cooperativity of the transitions increased with chain-length. It seems that the minimum half-width was reached with di-15:0-PC, possibly because at this length there was an optimal hydrophobic match with the 16:0-SM.

In order to assess molecular requirements for ideal miscibility, phase diagrams were prepared from the on- and off-set temperatures of the gel to liquid-crystalline phase transition, as described in Section 2. Fig. 4 shows the phase diagrams based on data recorded with binary mixtures of 16:0-SM and di-13:0-PC, di-14:0-PC, or di-15:0-PC. The phase diagram for di-16:0-PC/16:0-SM bilayers was published earlier [17]. A comparison of the phase diagrams based on experimental data and the simulated ideal phase diagrams allowed us to assess how well the different PC molecules mixed with 16:0-SM. From the obtained data with the symmetric chain PCs, it seems that di-14:0-PC

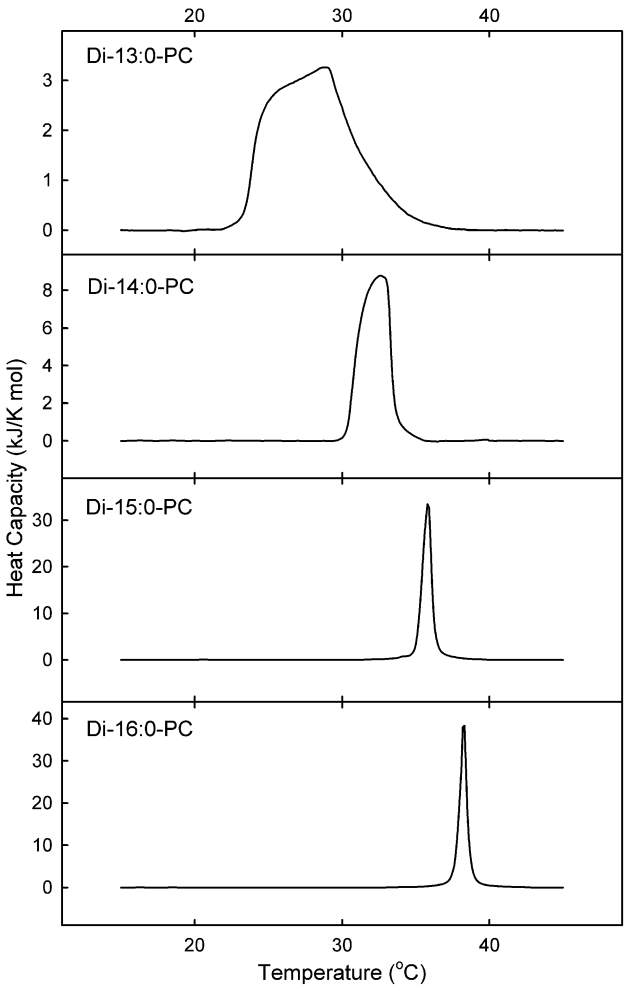


Fig. 2. Representative thermograms of binary mixtures of 16:0-SM and diacyl-PCs. Membranes composed of 16:0-SM and different symmetric PCs (X_{SM}=0.5) were heated with a rate of 0.5 °C/min.

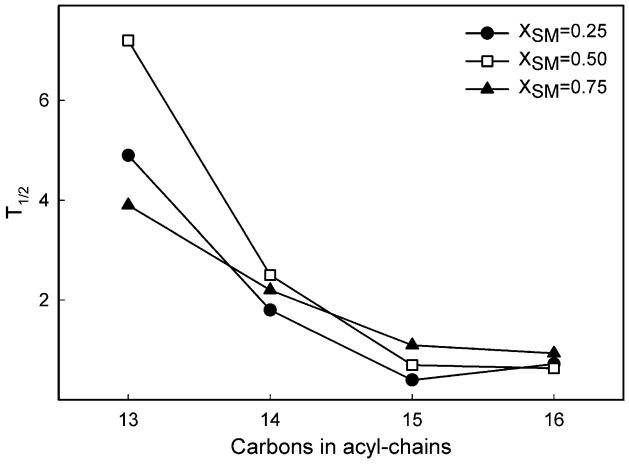


Fig. 3. The cooperativity of the main transition in mixtures of diacyl-PC and 16:0-SM. Phospholipid membranes with varying composition were heated at a rate of 0.5 °C/min. The cooperativity was assessed by measuring the half-width of the transition peaks. Panel A shows the half-widths as a function X_{SM}, and panel B shows the half-widths of the binary mixtures as a function of acyl-chain length.

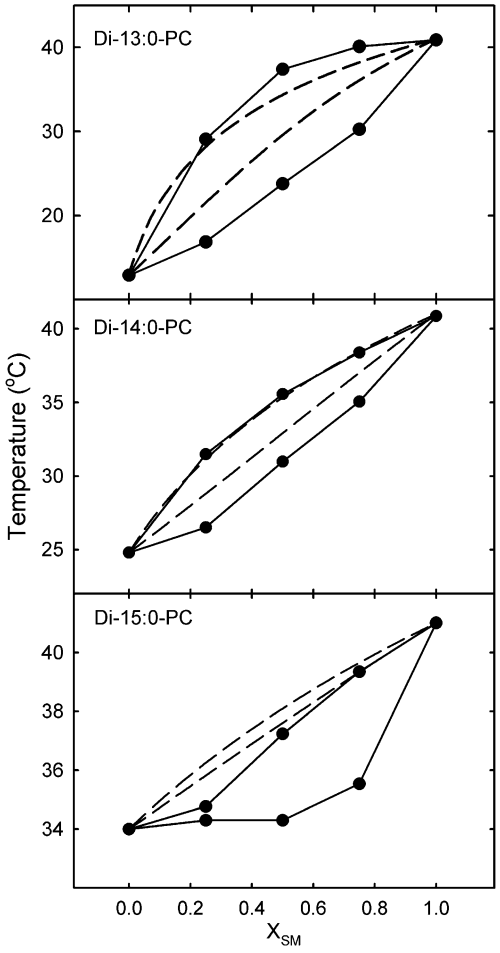


Fig. 4. Binary phase diagrams of binary mixtures of diacyl-PC and 16:0-SM. The phase diagrams were constructed based on DSC data as described in the text. The dashed lines are ideal phase diagrams calculated using Eqs. (1) and (2). The acyl-chain compositions of the PCs are denoted in the figure.

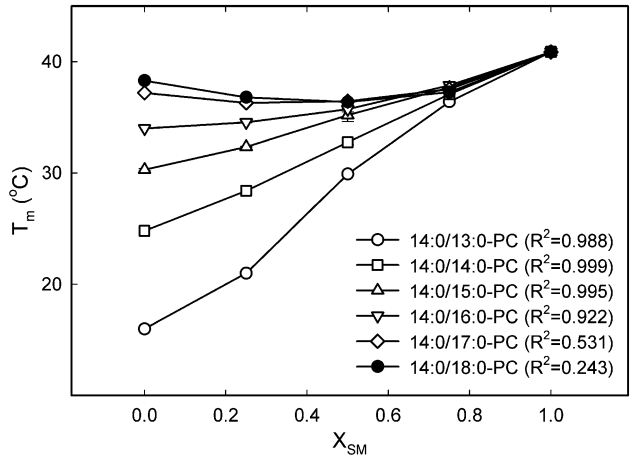


Fig. 5. The main phase transition temperatures of binary mixtures of asymmetric PC and 16:0-SM. Phospholipid membranes composed of 16:0-SM and PC, with a 14-carbon-long *sn*-1 chain and a 13–18-carbon-long *sn*-2 chain, were heated at a rate of 0.5 °C/min. The linearity of the T_m functions was evaluated by performing a linear fit on the data. The *R*-squared values from these fits are shown in the graph.

showed the best miscibility with 16:0-SM, because with this lipid, the observed phase diagram was closest to the diagram for ideal miscibility.

3.2. Miscibility of asymmetric saturated PC molecules with 16:0-SM

The results from the studies of symmetric diacyl-PC and 16:0-SM miscibility suggested that the di-14:0-PC mixed most favorably with 16:0-SM. To obtain more information of PC/SM mixing, we prepared a series of PC molecules with a saturated myristoyl chain in the *sn*-1 position and different saturated chains (13 to 18 carbons) in the *sn*-2 position. The interactions between asymmetric PCs and

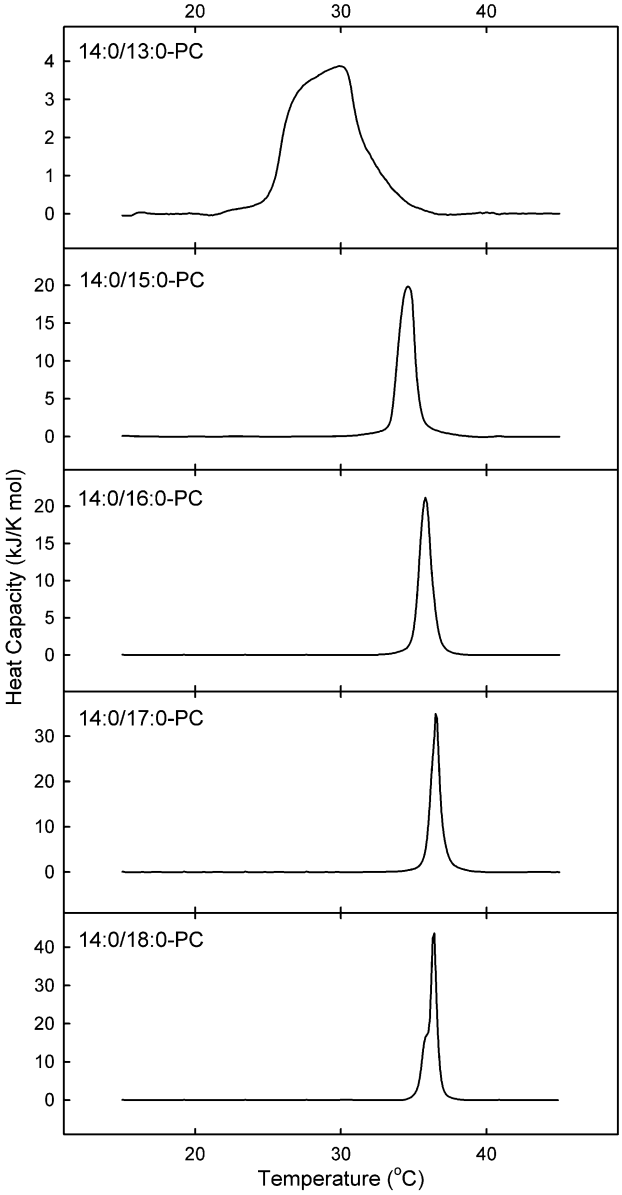


Fig. 6. Representative thermograms of binary mixtures of SM and asymmetric PCs. Membranes composed of 16:0-SM and different asymmetric PCs (X_{SM}=0.5) were heated at a rate of 0.5 °C/min.

16:0-SM were analyzed using the same DSC approach as with the symmetric diacyl-PCs. In Fig. 5 the T_m recorded from these experiments is shown as a function of the SM molar fraction. In membranes containing PCs with an *sn*-2 chain shorter than 16 carbons, the T_m increased fairly linearly with X_{SM}, whereas with all the longer PC molecules, the T_m did not increase linearly with X_{SM}, possibly indicating less favorable miscibility.

As observed with symmetric PCs, the cooperativity of the gel to liquid-crystalline phase transitions depended on the acyl-chain composition, as can be seen Fig. 6. The cooperativity of the phase transitions is shown as the half-widths of the gel to liquid-crystalline phase transitions in Fig. 7. As shown previously with the diacyl-PC molecules, the $T_{1/2}$ of the transition decreased with the chain length and eventually reached a plateau at a certain acyl-chain length. With the 14:0/X-PCs, the plateau was reached with *sn*-2 chain lengths of 16 carbons. In binary mixtures of 16:0-SM and 14:0/18:0-PC, the phase transition peak clearly indicated phase separation, although the total cooperativity of the peak was as low as in membranes composed of 14:0/16:0-PC and 16:0-SM. Based on this data (Figs. 6 and 7), it seems that 14:0/16:0-PC and 14:0/17:0-SM were the best match for 16:0-SM from a lateral packing point of view.

Most gel to liquid-crystalline phase transitions recorded with asymmetric PC molecules showed a single symmetric peak. The exceptions were binary mixtures of 16:0-SM and 14:0/13:0-PC or 14:0/18:0-PC, which resulted in thermograms that clearly contained several components (see Fig. 6). The most plausible explanation for the asymmetric phase transition peaks is some degree of phase separation.

As with the symmetric diacyl-PCs, the mixing of PC with SM was further analyzed by preparing phase diagrams and comparing the experimental data with simulated ideal phase

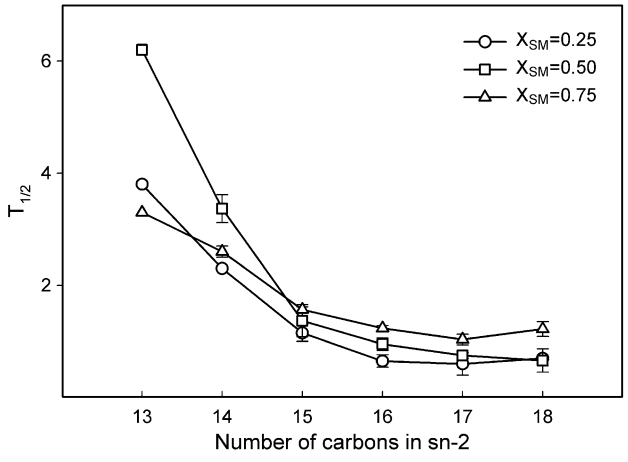


Fig. 7. The cooperativity of the main transition in mixtures of asymmetric PC and 16:0-SM. Phospholipid membranes composed of 16:0-SM and PC, with a 14-carbon-long *sn*-1 chain and a 13–18-carbon-long *sn*-2 chain, were heated at a rate of 0.5 °C/min. The cooperativity was assessed by measuring the half-width of the transition peaks. Panel A shows the half-widths as a function X_{SM}, and panel B shows the half-widths of the binary mixtures as a function of acyl-chain length.

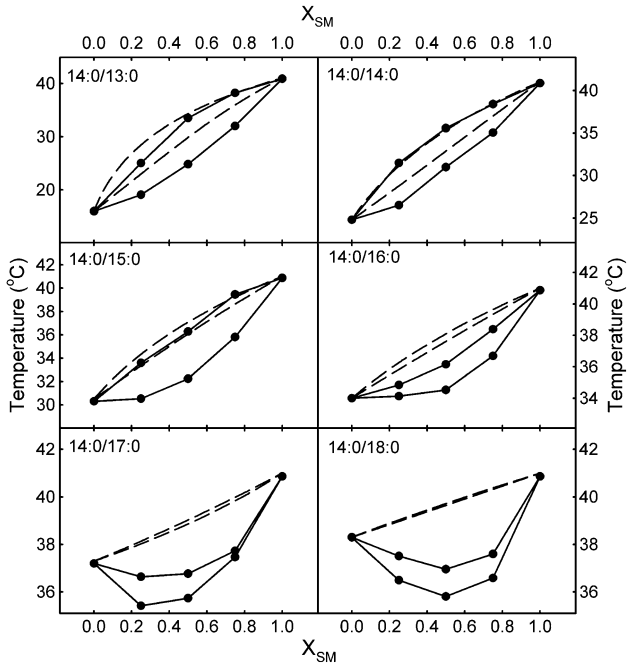


Fig. 8. Binary phase diagrams of binary mixtures of asymmetric PC and 16:0-SM. The phase diagrams were constructed based on DSC data as described in the text. The dashed lines are ideal phase diagrams calculated using Eqs. (1) and (2). The acyl-chain compositions of the PCs are denoted in the figure.

diagrams. All phase diagrams are shown in Fig. 8. The PC molecules with *sn*-2 acyl-chain lengths between 13 and 15 carbons gave phase diagrams which most closely matched the phase diagram of ideal mixing. When the *sn*-2 chain length increased (16–18 carbons), the observed phase diagrams deviated increasingly from the diagrams of ideal mixing. Out of the tested PCs, di-14:0-PC appeared to be the one that mixed most ideally with 16:0-SM (Fig. 8).

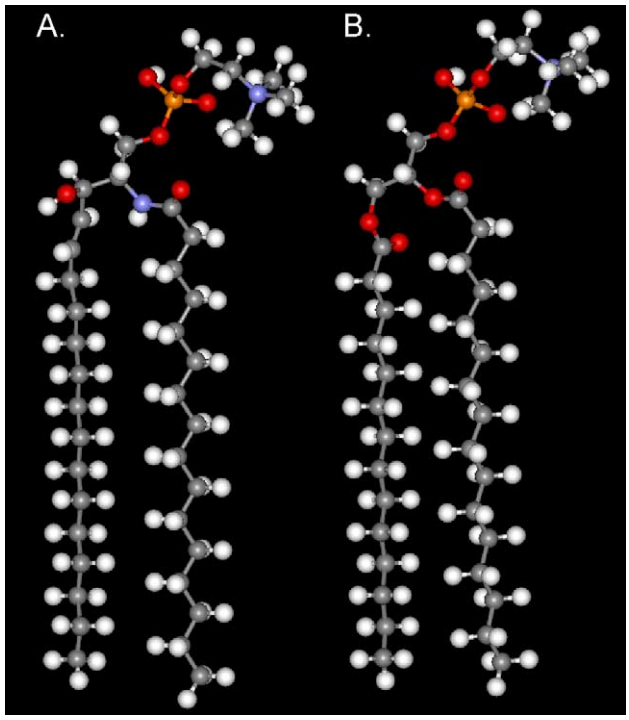
4. Discussion

Natural SM has longer and, on average, more saturated acyl-chains than PC [18]. Therefore, segregation into more ordered SM-rich domains and less ordered PC-rich domains occurs at lower temperatures (below the T_m of SM), but at higher temperatures (above the T_m for SM and PC) they are completely miscible [9]. This means that the interactions between SM and PC are temperature- and composition-dependent. In this study, we have looked at PC molecules with symmetric and asymmetric saturated acyl-chains. Most of the PCs examined were miscible with 16:0-SM both in the liquid-crystalline and the gel state. The exceptions were di-12:0-PC, di-13:0-PC, 14:0/13:0-PC and 14:0/18:0-PC, which showed evidence of phase separation (di-12:0-PC data not shown; Figs. 2 and 6). This was expected, since similar experiments with binary mixtures of PC molecules with similar differences in acyl-chain composition have also resulted in phase separation [19–21].

The literature contains several studies on the thermotropic behavior of PC/SM mixtures [9–12,17,22].These studies, however, offer limited information about how the interactions between SM and PC are affected by the acyl-chain composition. Through the use of a series of different acyl-chain defined lipids, it is possible to get more detailed information on how PC and SM molecules interact and organize in mixed bilayers. As expected, the results clearly show that the interactions between PC and SM molecules depend on the acyl-chain composition in SM and PC, respectively.

Information about the different PC/SM mixtures was obtained by comparing the T_m, the cooperativity, and the phase diagrams of the mixtures as a function of X_{SM}. From these parameters it was possible to determine how well 16:0-SM mixed with PCs with different acyl-chain composition. Addition of 16:0-SM to PC membranes composed of shorter chain PCs increased T_m, whereas inclusion of 16:0-SM to bilayers composed of longer chain PCs (di-16:0-PC, 14:0/17-PC and 14:0/18:0-PC) decreased the T_m (see Figs. 1 and 4). Since these longer-chain PCs have rather similar T_m as 16:0-SM, the plots of T_m vs. X_{SM} are almost horizontal lines (as could be expected). However, our interpretation of the fact that there was a depression in T_m upon inclusion of 16:0-SM is that the interactions between these PCs and 16:0-SM were less favorable than when the T_m was increased upon SM inclusion. The T_m functions of the binary PC/SM mixtures, as well as the phase diagrams based on the DSC data, indicated that di-14:0-PC mixed the most ideally with 16:0-SM, of all the PCs used in this study. This is interesting, since there is a large difference between T_m for 16:0-SM and di-14:0-PC bilayers (~17 °C). Di-16:0-PC, which has a similar T_m as 16:0-SM, mixed much less ideally with 16:0-SM than di-14:0-PC. Therefore, it is clear that ideal miscibility of PCs with 16:0-SM did not correlate with the individual T_m of phospholipid species. It is, however, not surprising that T_m does not correlate solely with the hydrophobic length of the molecules, since interfacial hydrogen bonding is also known to markedly affect the T_m (e.g., compare the T_m of 16:0-SM and 16:0-galactosylcerebroside, 41 and 80 °C, respectively, [17,23]).

The highest cooperativity of the main transition was observed in binary mixtures containing PCs with an average acyl-chain length ≥15 carbons, but in binary mixtures of 16:0-SM and 14:0/18:0-PC multiple peaks occurred, indicating phase separation. The glycerol backbone in glycerolipids has a similar configuration as the first three carbons in the sphingoid base of sphingolipids [24]. Therefore, it has been suggested that PCs with a 14:0 acyl-chain in *sn*-1 position would have a similar hydrocarbon length as the sphingoid base in SM [25,26]. The amide-linked acyl-chain in sphingolipids and the ester-linked *sn*-2 acyl-chain in glycerolipids are thought to have a rather similar configuration [26]. Accordingly, the 14:0/16:0-PC could be expected to resemble 16:0-SM in hydrophobic length (Scheme 1). Analyses of bilayer thickness have shown that addition of 16:0-SM to di-16:0-PC decreases the bilayer thickness and addition of 16:0-SM to di-14:0-PC bilayers increases the bilayer thickness [12,27]. This suggests that the effective length of 16:0-SM in a bilayer is closely matched by di-15:0-PC, or 14:0/16:0-PC. In the present study, the main transition of the binary PC/SM bilayers reached the highest cooperativity with di-15:0-PC (symmetric PCs) and 14:0/16:0-PC (asymmetric PCs), and phase separation was observed with 14:0/18:0-PC. We suggest that at these acyl-chain lengths the effective lengths of the PCs were similar to that of 16:0-SM, which would be in agreement with what is known about molecular configurations and bilayer thickness [11,24,26,27].



Scheme 1. Schematic picture showing the structures of 16:0-SM (A) and 14:0/16:0-PC (B).

Phospholipids, in which the effective lengths of the *sn*-1 and *sn*-2 acyl-chain differ, can form interdigitated bilayers [28]. In bilayers consisting of different phospholipids, mixing of the lipids can be influenced by interdigitation. Assuming that the interfacial conformations of PCs and SMs are similar, one could expect that 14:0/16:0-PC would have a similar effective chain length difference as 16:0-SM, while the other PCs in this study would not. Therefore, it could be expected that 14:0/16:0-PC would interact more favorably with 16:0-SM than the other PCs used in the study. Possibly the conformation of the PCs and SM might be different in mixed PC/SM bilayers than in pure PC or SM bilayers [29], thereby affecting the inter-leaflet acyl-chain interactions. The difference in effective chain length in the lipids used in this study was rather small, and therefore we think interdigitation could have had only a minor effect on

the bilayer organization in this study. There is, however, most likely an interrelation between inter-leaflet acyl-chain organization and molecular conformation and organization in the interfacial region. Possibly the phase separation observed in 16:0-SM/14:0/18:0-PC bilayers could be caused by interdigitation, although it has been proposed that 14:0/18:0-PC forms bilayers that are comparable to symmetric PCs [30]. However, NMR studies have shown that the conformation of the *sn*-2 carbonyl group is different in 14:0/18:0-PC and 14:0/16:0-PC [17].

Since the hydrogen bonding properties of SM and PC are quite different [7], it seems possible that membranes containing both PC and SM can have a different interfacial hydrogen bonding network compared to membranes of the pure phospholipids. In an infrared spectroscopy study of membranes composed of di-16:0-PC and egg SM, small changes in the frequencies of the C=O groups (both *sn*-1 and *sn*-2) of PC and of the amide group of SM were observed in comparison to pure di-16:0-PC [29]. The authors concluded that this change most likely resulted from conformational changes in the polar region of the lipids rather than from changes in the hydrogen bonding network. However, the results did not exclude hydrogen bonding between the phosphate group of PC and the OH-group of SM [29]. In the present study, it was observed that the interactions between PC and SM are clearly dependent on the acyl-chain composition, and di-16:0-PC was shown to mix far from ideally with 16:0-SM. We assume that the hydrogen bonding within and between molecules and their conformational changes were energetically more favorable in those PC/SM bilayers that mixed more ideally. Further, there is evidence that SM and PC bilayers also differ in hydration [31–33]. It is possible that PCs with different acyl-chain lengths could affect the hydration of 16:0-SM/PC bilayers differently, affecting the T_m and the ideality of the mixture. The fact that 16:0-SM mixed more ideally with di-14:0-PC than with other PCs thought to match 16:0-SM more closely in effective length (based on, e.g., molecular modeling) suggests that the ideality of mixing is markedly dependent on molecular interactions in the membrane–water interface.

The ideality of mixing of PC and SM molecules could further be affected by the thermotropic properties of the PC and SM components. Within the temperature range of the DSC measurements, di-16:0-PC, for example, exists in three different phase states: the lamellar gel phase, the rippled gel phase, and the liquid-crystalline phase [34]. Within the same temperature range, 16:0-SM has also been observed to exist in the same three phase states [10]. On the other hand, 14:0/16:0-PC has been shown to have no lamellar gel phase, and the pre-transition found in pure 14:0/16:0-PC membranes is suggested to arise from a sub to rippled gel phase transition [20]. The thermotropic phase behavior of PCs has been shown to be affected by interdigitation [35,36], which could explain the different phase behavior and conformations of these PCs. Possibly the thermotropic phase behavior of the phospholipids affects their miscibility to some extent, but clearly more studies are needed to elucidate this possibility.

One of the objectives in this study was to contribute to the discussion of which PC/SM pair should be used in studies where the properties of the two phospholipids are compared. The importance of the acyl-chain composition has for example been observed in studies of cholesterol-phospholipid interactions [13,14]. However, there is no direct evidence for which PC/SM pair one should compare in such studies. The PC that most often has been compared to 16:0-SM is di-16:0-PC [17,29,37–41]. According to the present study and previously published results [12,17], di-16:0-PC is apparently not the best choice. Rather, it seems that di-15:0-PC or 14:0/16:0-PC is a better choice based upon the results in this study and previously published results [12,27]. Di-14:0-PC was the PC that mixed most ideally with 16:0-SM, but based on the lower cooperativity, and the slightly asymmetrical shape of the transition peaks of di-14:0-PC/16:0-SM mixtures, we do not consider it the PC that is best comparison to 16:0-SM.

In conclusion, this study showed that the miscibility of PC and SM had a clear dependence on the acyl-chain lengths in SM and PC. The PC and SM that mixed most ideally were di-14:0-PC and 16:0-SM, but the highest cooperativity was reached with longer acyl-chains. Further, the results emphasized the importance of considering acyl-chain composition in studies where the properties of PC and SM are compared.

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References

[1] Y. Barenholz, T.E. Thompson, Sphingomyelin: biophysical aspects, Chem. Phys. Lipids 102 (1999) 29–34.
[2] M. Koval, R.E. Pagano, Intracellular transport and metabolism of sphingomyelin, Biochim. Biophys. Acta 1082 (1991) 113–125.
[3] K. Simons, E. Ikonen, Functional rafts in cell membranes, Nature 387 (1997) 569–572.
[4] 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.
[5] D.A. Brown, E. London, Functions of lipid rafts in biological membranes, Annu. Rev. Cell Dev. Biol. 14 (1998) 111–136.
[6] H. Ohvo-Rekila, B. Ramstedt, P. Leppimaki, J.P. Slotte, Cholesterol interactions with phospholipids in membranes, Prog. Lipid Res. 41 (2002) 66–97.
[7] B. Ramstedt, J.P. Slotte, Membrane properties of sphingomyelins, FEBS Lett. 531 (2002) 33–37.
[8] J.M. Boggs, Intermolecular hydrogen bonding between lipids: influence on organization and function of lipids in membranes, Can. J. Biochem. 58 (1980) 755–770.

[9] S.H. Untrach, G.G. Shipley, Molecular interactions between lecithin and sphingomyelin. Temperature- and composition-dependent phase separation, J. Biol. Chem. 252 (1977) 4449–4457.
[10] 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.
[11] P.R. Maulik, G.G. Shipley, Interactions of *N*-stearoyl sphingomyelin with cholesterol and dipalmitoylphosphatidylcholine in bilayer membranes, Biophys. J. 70 (1996) 2256–2265.
[12] P.R. Maulik, G.G. Shipley, *N*-Palmitoyl sphingomyelin bilayers: structure and interactions with cholesterol and dipalmitoylphosphatidylcholine, Biochemistry 35 (1996) 8025–8034.
[13] T.P.W. McMullen, R.N.A.H. Lewis, R.N. McElhaney, Differential scanning calorimetric study of the effect of cholesterol on the thermotropic phase behavior of a homologous series of linear saturated phosphatidylcholines, Biochemistry 32 (1993) 516–522.
[14] B. Ramstedt, J.P. Slotte, Interaction of cholesterol with sphingomyelins and acyl-chain-matched phosphatidylcholines: a comparative study of the effect of the chain length, Biophys. J. 76 (1999) 908–915.
[15] M.A. Kaluzny, L.A. Duncan, M.V. Merrit, D.E. Epps, Rapid separation of lipid classes in high yield and purity using bonded phase columns, J. Lipid Res. 26 (1985) 135–140.
[16] S. Mabrey, J.M. Sturtevant, Investigation of phase transitions of lipids and lipid mixtures by sensitivity differential scanning calorimetry, Proc. Natl. Acad. Sci. U. S. A. 73 (1976) 3862–3866.
[17] T.K. Nyholm, M. Nylund, J.P. Slotte, A calorimetric study of binary mixtures of dihydrosphingomyelin and sterols, sphingomyelin, or phosphatidylcholine, Biophys. J. 84 (2003) 3138–3146.
[18] Y. Barenholz, T.E. Thompson, Sphingomyelins in bilayers and biological membranes, Biochim. Biophys. Acta 604 (1980) 129–158.
[19] C. Huang, J.T. Mason, Structure and properties of mixed-chain phospholipid assemblies, Biochim. Biophys. Acta 864 (1986) 423–470.
[20] B.A. Lewis, S.K. Das Gupta, R.G. Griffin, Solid-state NMR studies of the molecular dynamics and phase behavior of mixed-chain phosphatidylcholines, Biochemistry 23 (1984) 1988–1993.
[21] P. van Dijck, A. Kaper, H. Oonk, J. de Gier, Miscibility properties of binary phosphatidylcholine mixtures. A calorimetric study, Biochim. Biophys. Acta 470 (1977) 58–69.
[22] B.R. Lentz, M. Hoechli, Y. Barenholz, Acyl chain order and lateral domain formation in mixed phosphatidylcholine-sphingomyelin multilamellar and unilamellar vesicles, Biochemistry 20 (1981) 6803–6809.
[23] K. Saxena, R.I. Duclos, P. Zimmermann, R.R. Schmidt, G.G. Shipley, Structure and properties of totally synthetic galacto- and glucocerebrosides, J. Lipid Res. 40 (1999) 839–849.
[24] I. Pascher, M. Lundmark, P.G. Nyholm, S. Sundell, Crystal structures of membrane lipids, Biochim. Biophys. Acta 1113 (1992) 339–373.
[25] J.M. Holopainen, J. Lemmich, F. Richter, O.G. Mouritsen, G. Rapp, P.K. Kinnunen, Dimyristoylphosphatidylcholine/C16:0-ceramide binary liposomes studied by differential scanning calorimetry and wide- and small-angle X-ray scattering, Biophys. J. 78 (2000) 2459–2469.
[26] X.M. Li, M.M. Momsen, J.M. Smaby, H.L. Brockman, R.E. Brown, Cholesterol decreases the interfacial elasticity and detergent solubility of sphingomyelins, Biochemistry 40 (2001) 5954–5963.
[27] W.I. Calhoun, G.G. Shipley, Sphingomyelin-lecithin bilayers and their interaction with cholesterol, Biochemistry 18 (1979) 1717–1722.
[28] J.L. Slater, C.H. Huang, Interdigitated bilayer membranes, Prog. Lipid Res. 27 (1988) 325–359.
[29] J. Villalain, A. Ortiz, J.C. Gomez-Fernandez, Molecular interactions between sphingomyelin and phosphatidylcholine in phospholipid vesicles, Biochim. Biophys. Acta 941 (1988) 55–62.
[30] J.T. Mason, C. Huang, R.L. Biltonen, Calorimetric investigations of saturated mixed-chain phosphatidylcholine bilayer dispersions, Biochemistry 20 (1981) 6086–6092.
[31] S.W. Chiu, S. Vasudevan, E. Jakobsson, R.J. Mashl, H.L. Scott, Structure of sphingomyelin bilayers: a simulation study, Biophys. J. 85 (2003) 3624–3635.
[32] G.L. Jendrasiak, R.L. Smith, The effect of the choline head group on phospholipid hydration, Chem. Phys. Lipids 113 (2001) 55–66.
[33] T.J. McIntosh, S.A. Simon, D. Needham, C. Huang, Interbilayer interactions between sphingomyelin and sphingomyelin/cholesterol bilayers, Biochemistry 31 (1992) 2020–2024.
[34] T.P.W. McMullen, R.N. McElhaney, New aspects of the interaction of cholesterol with dipalmitoylphosphatidylcholine bilayers as revealed by high-sensitivity differential scanning calorimetry, Biochim. Biophys. Acta 1234 (1995) 90–98.
[35] J. Mattai, P.K. Sripada, G.G. Shipley, Mixed-chain phosphatidylcholine bilayers: structure and properties, Biochemistry 26 (1987) 3287–3297.
[36] J.T. Mason, R.E. Cunningham, T.J. O'Leary, Lamellar-phase polymorphism in interdigitated bilayer assemblies, Biochim. Biophys. Acta 1236 (1995) 65–72.
[37] S.N. Ahmed, D.A. Brown, E. London, On the origin of sphingolipid/cholesterol-rich detergent-insoluble cell membranes: physiological concentrations of cholesterol and sphingolipid induce formation of a detergent-insoluble, liquid-ordered lipid phase in model membranes, Biochemistry 36 (1997) 10944–10953.
[38] T. Bystrom, G. Lindblom, Molecular packing in sphingomyelin bilayers and sphingomyelin/phospholipid mixtures, Spectrochim. Acta, A: Mol. Biomol. Spectrosc. 59 (2003) 2191–2195.
[39] P. Mattjus, J.P. Slotte, Does cholesterol discriminate between sphingomyelin and phosphatidylcholine in mixed monolayers containing both phospholipids? Chem. Phys. Lipids 81 (1996) 69–80.
[40] T. Nyholm, M. Nylund, A. Soderholm, J.P. Slotte, Properties of palmitoyl phosphatidylcholine, sphingomyelin, and dihydrosphingomyelin bilayer membranes as reported by different fluorescent reporter molecules, Biophys. J. 84 (2003) 987–997.
[41] B.Y. van Duyl, D. Ganchev, V. Chupin, B. de Kruijff, J.A. Killian, Sphingomyelin is much more effective than saturated phosphatidylcholine in excluding unsaturated phosphatidylcholine from domains formed with cholesterol, FEBS Lett. 547 (2003) 101–106.