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Effect of hydrophobic mismatch and interdigitation on sterol/sphingomyelin interaction in ternary bilayer membranes☆

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ABSTRACT

Sphingomyelin (SM) is a major phospholipid in most cell membranes. SMs are composed of a long-chain base (often sphingosine, 18:1$^{\Delta 4t}$), and *N*-linked acyl chains (often 16:0, 18:0 or 24:1$^{\Delta 15c}$). Cholesterol interacts with SM in cell membranes, but the acyl chain preference of this interaction is not fully elucidated. In this study we have examined the effects of hydrophobic mismatch and interdigitation on cholesterol/sphingomyelin interaction in complex bilayer membranes. We measured the capacity of cholestatrienol (CTL) and cholesterol to form sterol-enriched ordered domains with saturated SM species having different chain lengths (14 to 24 carbons) in ternary bilayer membranes. We also determined the equilibrium bilayer partitioning coefficient of CTL with 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) membranes containing 20 mol% of saturated SM analogs. Ours results show that while CTL and cholesterol formed sterol-enriched domains with both short and long-chain SM species, the sterols preferred interaction with 16:0-SM over any other saturated chain length SM analog. When CTL membrane partitioning was determined with fluid POPC bilayers containing 20 mol% of a saturated chain length SM analog, the highest affinity was seen with 16:0-SM (both at 23 and 37 °C). These results indicate that hydrophobic mismatch and/or interdigitation attenuate sterol/SM association and thus affect lateral distribution of sterols in the bilayer membrane.

1. Introduction

Sphingomyelin (SM) is a phosphosphingolipid commonly found in the cell membranes of most cells [1]. It is also present in low density lipoproteins [2]. The structure of SM molecular species vary. Mostly SM contain sphingosine (18:1$^{\Delta 4t}$) as the long-chain base [3], but other bases are also found in biologically relevant SM species [4]. Of the *N*-linked acyl chains, 16:0, 18:0 and 24:1$^{\Delta 15c}$ are the most common [1,3], although many other acyl chain lengths have been observed in natural SM species. Sometimes the long-chain bases or the acyl chains are methyl-branched, and/or hydroxylated [5,6]. Since SM molecular species often have saturated acyl chains, they confer high degree of order to the hydrophobic region of bilayer membranes [7–10].

Membranes enriched in SM are known to also be enriched in cholesterol [11], and many studies have shown that SM is an important solubilizer of cholesterol [12–14]. In fact, when membranes contain acyl-chain matched SMs and phosphatidycholines (PC), cholesterol appear to favor interaction with SM over PC [9,15–18]. The sterol/phospholipid interactions are affected by many different parameters, such as phospholipid head-group size and charge [19,20], acyl chain length and degree of unsaturation [18], acyl-chain hydroxylation [21] or methyl branching [22], just to name a few. Sterol/phospholipid interactions are naturally also affected by changes in sterol structure [23–25]. Domain formation in and phase behavior of ternary bilayer systems containing unsaturated PC, saturated SM, and cholesterol has been the subject of several studies in recent years, and the obtained data have importantly increased our understanding of cholesterol's interactions with PCs and SMs [26–28]. The acyl-chain length dependence of sterol/phospholipid interaction has been addressed in only a few studies over the years. For bilayers with symmetric saturated PCs, DSC studies have revealed that cholesterol interaction is most favorable (or least unfavorable) with di-17:0-PC, and less favorable with shorter or longer chain PCs [29]. In PC monolayer membranes, androsterol desorption rate was slowest from di-14:0-PC and higher from monolayers with shorter or longer PC species [30], again suggesting that sterol/PC interaction is most favorable at a certain match between the sterol length and the neighboring PC acyl chain length. Obviously the optimal acyl chain length is different for cholesterol and androsterol. When cholesterol desorption rates were measured from monolayer membranes containing asymmetric PC or SM species with matched *sn*-2 or *N*-linked acyl chains, cholesterol desorption rates were markedly influenced by the PC *sn*-2 chain length, but not by the SM *N*-linked chain length [18]. It was speculated that hydrophobic mismatch between PC and

Abbreviations: 7SLPC, 1-palmitoyl-2-stearoyl-(7-doxyl)-*sn*-glycero-3-phosphocholine; CTL, cholesta-5,7 (11)-trien-3-beta-ol; DPH, 1,6-diphenyl-1,3,5-hexatriene; K_x, partitioning coefficient; PC, phosphatidylcholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; SM, sphingomyelin

☆ We dedicate this paper to professor Chezy Barenholz on the occasion of his 70th birthday.

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cholesterol led to a significant lateral segregation of cholesterol, which could explain the desorption data. Apparently, cholesterol did not behave similarly in monolayers containing mismatched SMs, since desorption rates were mostly unaffected by hydrophobic mismatch. Our laboratory has recently also reported that sterols in ternary bilayer systems may form ordered domains with different SM species with variable acyl chain length or unsaturation [9,21,31,32]. However, a systematic study on the chain-length dependence of sterol/SM interaction has not been reported.

Asymmetry in phospholipid structure (i.e. different hydrophobic length of the two aliphatic residues) does not affect lateral packing and possible lipid interactions similarly in monolayer and bilayer membranes. Long-chain SMs are known to form interdigitated packing arrangements in bilayer membranes, which are not observed in monolayer membranes [33,34]. Since the effect of interdigitation on e.g., cholesterol/SM interaction in complex bilayer membranes has not been systematically examined, we undertook to study the lateral interaction of cholesterol with fluid and ordered phospholipids in bilayer membranes. The fluid lipid was chosen to be 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), and the ordered phospholipid was SM with varying degree of chain mismatch (or molecular asymmetry). Cholesterol was used as the bulk sterol, and cholesta-5,7,9-trien-3 beta-ol (at 1 mol%; CTL) was our cholesterol mimic, whose lateral distribution and bilayer partitioning was directly measured. We and others have previously shown that CTL is the best fluorescent cholesterol mimic available [17,35–37], and although CTL is slightly more polar than cholesterol, its relative membrane partitioning into different phospholipid bilayer is very similar to that observed for e.g., radiolabeled cholesterol [36–38].

2. Materials and methods

2.1. Materials

Highly pure POPC and D-*erythro*-sphingosyl phosphorylcholine were purchased from Avanti Polar Lipids (Alabaster, AL, USA) and used without further purification. Saturated fatty acids used for SM synthesis were obtained from Larodan Fine Chemicals (Malmö Sweden). 16:0-SM was isolated from egg SM, 18:0-SM from bovine brain SM, and 22:0-SM from milk SM using reverse-phase HPLC [22]. These molecular species had sphingosine ($18{:}1^{\Delta 4t}$) as the long-chain base. All other SM analogs (14:0-SM, 15:0-SM, 17:0-SM, 19:0-SM, 20:0-SM, 24:0-SM) were synthesized from the D-*erythro*-sphingosyl phosphorylcholine and fatty acids as described previously [22]. The identity of all purified SM analogs was verified by ESI-MS (purity better than 99%). Stock solutions of the lipids were prepared in hexane/2-propanol (3/2, by vol), except for stock solutions of 20:0-SM, 22:0-SM and 24:0-SM which were prepared in hexane/2-propanol containing 25 vol.% methanol. All the phospholipid stock solutions were taken to ambient temperature before use and the concentration of the lipids was determined by phosphate assay subsequent to total digestion by perchloric acid. Stock solutions of the phospholipids were stored at −20 °C.

1-Palmitoyl-2-stearoyl-(7-doxyl)-sn-glycero-3-phosphocholine (7SLPC) and CTL, which act as a quencher and a fluorophore, respectively, were synthesized and purified as described previously [22,39–41]. The identity of CTL was positively verified by APCI-MS. 1,6-Diphenyl-1,3,5-hexatriene (DPH) was purchased from Molecular Probes (Leiden, the Netherlands). The fluorophores were stored under argon in the dark at −87 °C until dissolved in argon-purged ethanol (CTL) or methanol (DPH). The concentration of CTL and DPH in the respective stock solutions was determined spectrophotometrically using their molar absorption coefficients (ε) values: 11,250 M^{-1} cm^{-1} at 324 nm for CTL, and 88,000 M^{-1} cm^{-1} at 350 nm for DPH. The stock solutions of the fluorophores were stored in the dark at −20 °C and used within a week. Water was used as aqueous solvent in all studies. All other inorganic and organic chemicals used were of the highest purity available. The solvents used were of spectroscopic grade. Water was purified by reverse osmosis followed by passage through a Millipore UF Plus water purification system having final resistivity of 18.2 MΩcm.

2.2. Preparation of vesicles

Lipid vesicles used in the study were prepared to a lipid concentration of 50 μM. Required amounts of the lipids and probes were mixed and the solvents were evaporated under a constant flow of N_2 at 35 °C. When preparing liposomes with mixed lipid compositions, the lipids were redissolved in chloroform to assure a homogeneous mixing of the lipids. Once the lipids were thoroughly mixed, the solvent was redried to yield a lipid film. After further drying under high vacuum for at least 1 h at room temperature, the lipid mixtures were hydrated by adding phosphate-buffered saline (pH 7.4). The temperature of solvent was kept above the gel-to-liquid crystalline phase transition temperature (T_m) of the lipid with the highest melting temperature before addition to the dry lipid film. The lipid suspension was maintained above T_m during the hydration period of 20 min. Samples were then vortexed to disperse the lipids in the buffer. For fluorescence measurements, multilamellar vesicles were prepared by probe sonication (sonicated for 2 min with 20% duty cycle and 15 W power output) using a Branson probe sonifier (W-450, Branson Ultrasonics, Danbury, CT, USA).

Unilamellar vesicles used for the CTL partitioning assay were prepared by extrusion (200 nm pore diameter) as described previously [36].

2.3. Steady-state fluorescence anisotropy measurements

The steady-state fluorescence anisotropy of DPH incorporated into lipid vesicles was measured on a T-format PTI Quanta-Master spectrofluorimeter (Photon Technology International, Lawrenceville, NJ, USA), essentially following the procedure described in [22]. The wave lengths of excitation and emission were 360 nm and 430 nm, respectively, and the DPH concentration was 1 mol%. The steady state anisotropy was calculated as described in [42].

2.4. Fluorescence quenching measurements

In order to follow the formation and melting of ordered domains, the steady-state quenching of CTL or DPH by 7SLPC was measured on a PTI Quanta-Master spectrofluorimeter, essentially as described in References [17,42]. The samples were heated from 10 °C to 70 °C at a rate of 5 °C/min. Fluorescence intensity of the fluorophores was detected with excitation and emission wavelengths at 324 nm and 374 nm for CTL (1 mol%) and at 360 nm and 430 nm for DPH (1 mol %), respectively. The fluorescence intensity was measured for both a F-sample (quenched) consisting of POPC/7SLPC/SM analog/cholesterol, (30:30:30:10, molar ratio) and a F_0 sample (unquenched), in which 7SLPC had been replaced with POPC. The fluorescence intensity for the *F* sample, *F*, was divided by the fluorescence intensity for the F_0 sample, F_0. The resultant F/F_0, which gives the fraction of unquenched CTL or DPH fluorescences, was plotted against temperature.

2.5. Sterol partitioning into unilamellar vesicles

The distribution of CTL between methyl-β-cyclodextrin (Sigma Chemicals, St. Louis, MO, USA) and extruded large unilamellar phospholipid vesicles (prepared in phosphate-buffered saline (pH 7.4); 200 nm pore diameter) was determined as described in References [36,37], a method significantly modified from the procedure reported by Niu and Litman [38]. The assay yields the molar fraction partition coefficient, K_x, for CTL. The CTL and thus sterol concentration was 2 mol%

in all partitioning experiments. A high K_x indicates a higher affinity of CTL for the bilayer as compared with methyl-β-cyclodextrin.

3. Results

3.1. DPH anisotropy in SM bilayer membranes

The T_m of the gel-to-liquid crystalline phase transition of synthetic racemic and *D-erythro*-SM analogs with varying saturated acyl chain lengths have been measured (mainly by DSC) by several groups in the past [43–48]. We measured a steady-state anisotropy of DPH incorporated into respective bilayers of the saturated SM analogs as a function of temperature. The resulting data gave information about both acyl chain order in the bilayer (anisotropy), and T_m values for the SM analogs. As shown in Fig. 1, the gel phase anisotropy values were almost the same (~0.35) for all the SM analogs with varying acyl chain lengths. As the temperature was allowed to increase, the SM bilayers underwent a gel-to-liquid crystalline phase transition at a specific T_m for each SM analog. In the fluid state, the DPH measured anisotropies also were rather similar ($<r>$ 0.13 ± 0.01) at a temperature of T_m + 10 °C (Fig. 1) for the different acyl chain analogs of SM, suggesting that chain-length mismatch did not markedly affect acyl chain order of this phase. When *T*m values obtained by the anisotropy measurement were plotted as a function of the acyl chain length, as shown in Fig. 2, they (filled symbols) increased with increasing the acyl chain length, but not linearly. Thus, the shorter acyl chain SM analogs (carbon number <16) showed a great increase in T_m with varying the chain length, which contrasted with a small change with the chain length observed for the longer acyl chain SM analogs. Very similar T_m values have been reported for similar SM analogs using DSC (open symbols, Fig. 2 [48]). Whereas symmetric PCs have a fairly linear relationship between T_m and chain length [29,49], this is clearly not the case for the asymmetric SMs.

3.2. Formation of ordered domains in ternary bilayer systems

A study on the interaction of sterol with the SM analogs was performed for a ternary bilayer system containing a fluid glycerophospholipid, POPC. Thus, we investigated how CTL was able to associate with the SM analogs and form ordered domains which enable the incorporated fluorescent-sterol to be protected from quenching by a fluid quencher, 7SLPC. The resulting *F*/*F*0 versus temperature curves are shown for the respective SM analogs in Fig. 3. We have previously

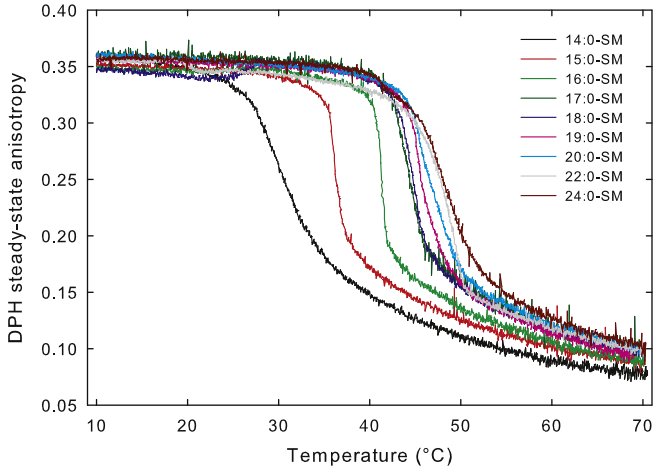

Fig. 1. Steady-state anisotropy of DPH in pure SM bilayers as a function of temperature. Experiments were performed with multilamellar vesicles at a scan rate of 2 °C/min with a SM concentration of 50 μM and 1 mol% of DPH. Saturated SM chain length analogs (14 to 24 carbons) were used. The graph show representative data from reproducible experiments.

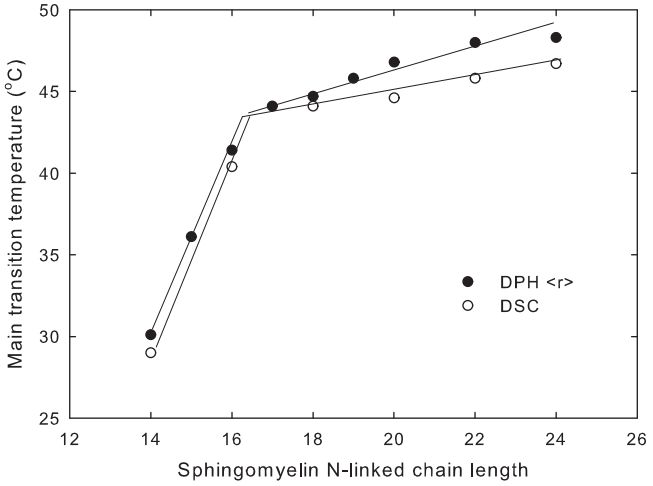

Fig. 2. DPH- and DSC-reported gel-phase melting in pure SM analog bilayers. The T_m for the gel-phase melting in SM analog bilayers was determined from the DPH anisotropy curves in Fig. 1 (filled symbols). Corresponding T_m values determined by DSC are shown as open symbols (DSC data taken from Reference [48]). Lines drawn were fitted by eye and are included for emphasis only.

shown that CTL, as well as cholesterol, forms a sterol-enriched ordered domain with the 16:0-SM in the ternary bilayer system [17]. CTL in the ordered domains was observed to be protected from quenching by 7SLPC until the domain began to melt at an increased temperature. During the melting of the ordered domains, the likelihood of interactions (i.e., quenching) between CTL and 7SLPC increases. The melting behavior for the ordered 16:0-SM-domains is shown in green in Fig. 3. As shown in Fig. 4, the difference in F/F_0 before and after the melting, denoted as $\Delta F/F_0$, is about as large as 0.35 for the 16:0-SM, indicating a larger amount of CTL present in the ordered 16:0-SM domain relative to the corresponding other SM analog domains. The $\Delta F/F_0$ is of course also affected by 7SLPC miscibility in the SM-rich

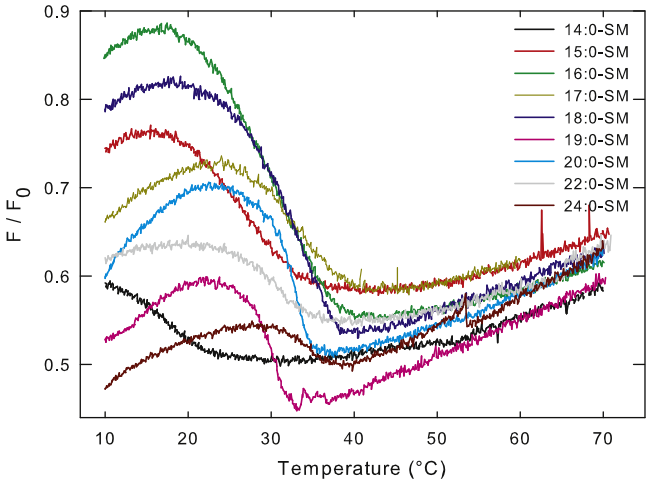

Fig. 3. Melting of ordered domains observed from the quenching of CTL fluorescence. The melting profile is shown as the *F*(quenched)/F_0(unquenched) ratio plotted versus temperature. The *F*-sample consisted of POPC/7SLPC/SM analog/cholesterol/CTL (30:30:30:9:1 mol%) and in the F_0-sample 7SLPC was replaced with POPC. The curves are representative from several experiments (n = 2–4). The measured approximate temperatures where 50% of the domains had melted were 18 °C (14:0-SM), 27 °C (15:0-SM), 30 °C (16:0-SM), 32 °C (17:0-SM), 33 °C (18:0-SM), 30 °C (19:0-SM), 33 °C (20:0-SM), 30 °C (22:0-SM) and 34 °C (24:0-SM). The approximate "end of melting"-temperatures were 24 °C (14:0-SM), 36 °C (15:0-SM), 40 °C (16:0-SM), 40 °C (17:0-SM),40 °C (18:0-SM), 34 °C (19:0-SM), 36 °C (20:0-SM). 40 °C (22:0-SM) and 39 °C (24:0-SM).

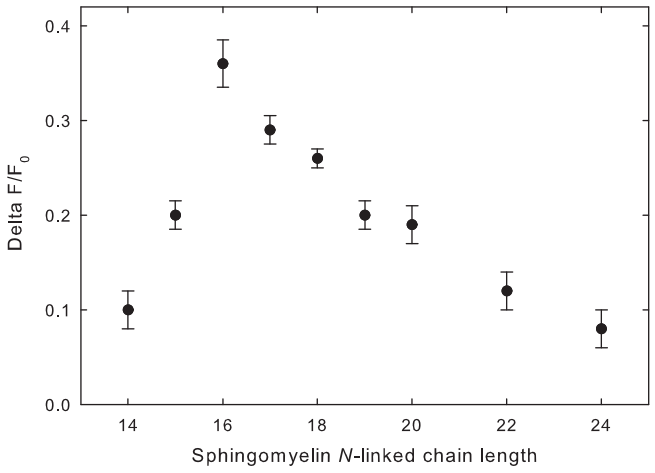

Fig. 4. Delta F/F_0 shown for different SM analogs. Delta F/F_0 is the difference in F/F_0 before and after melting of the ordered domains. Values are taken from CTL quenching experiments as shown in Fig. 3. Values are averages from 2 to 4 measurements ± S.E.M.

domain. When the property of CTL-enriched domains was compared for other SM analog systems, it was observed that the 14:0-SM formed the least stable domain (Fig. 3) and also the $\Delta F/F_0$ was small (Fig. 4). All other SM analogs formed sterol-enriched domains which were fully melted around 40 °C (for details about approximate melting temperatures measured from data in Fig. 3, please refer to the figure legend). However, the $\Delta F/F_0$ was largest for 16:0-SM, and was markedly lower for both shorter and longer SM analogs, suggesting that CTL interacted most favorably with 16:0-SM. When DPH quenching susceptibility was determined in ternary bilayer systems of POPC/SM/sterol, the ordered domain stability as reported by DPH was similar to that reported by CTL (data not shown).

3.3. Bilayer equilibrium partitioning of CTL

To study the affinity of sterols for phospholipid bilayers, one determines the equilibrium partitioning coefficient (K_x) for the sterols [36–38,50]. We measured the K_x of CTL in both a POPC bilayer and POPC/SM analog bilayers at two different temperatures of 23 and 37 °C. In Fig. 5, the results show clearly that CTL has a higher K_x to the bilayers containing SM analogs, as compared to the bilayer of POPC alone. This is consistent with our previous findings comparing CTL affinity between POPC and POPC/16:0-SM (4:1 by mol) bilayers [36]. The finding that all SM analogs were able to increase the K_x of CTL relative to the pure POPC bilayer further suggests that CTL was able to interact with all of the SM analogs studied. However, comparing K_x values of CTL for the bilayers containing different SM analogs revealed clearly that CTL displayed the highest affinity to the bilayer containing 16:0-SM, suggesting the most favorable interaction with this molecular species. This observation was true at both temperatures, although the relative differences were larger at 23 than at 37 °C.

4. Discussion

Sphingomyelins are the major lipids of plasma membranes which can have an asymmetric molecular shape due to the mismatch in hydrophobic length of the long sphingosine-chain relative to a long *N*-linked acyl chain (e.g., C24). In addition, a long *N*-linked acyl chain in SM is mismatched also with regard to cholesterol, and to adjacent glycerophospholipid acyl chains, thus having the potential to affect interlipid interactions in the membrane. We have in this study systematically examined how the length of the *N*-linked acyl chain in SM affect domain forming properties of SMs, and interactions with sterols.

One obvious consequence of hydrophobic mismatch in asymmetric SMs is the non-linear chain-length dependence of the T_m of the gel-to-liquid crystalline phase transition. Whereas symmetric saturated phospholipids obey a fairly linear relationship between chain length and T_m [29], asymmetric SMs fail to do so (Fig. 2). The gel phase apparently becomes destabilized in the mismatched SM bilayers by the increased likelihood of *gauche* rotamers in the terminal part of the *N*-linked acyl chain which extends beyond the length of the long-chain base [48]. It is also possible that interdigitation of the long-chain acyl chain into the opposing leaflet contribute to gel phase destabilization. X-ray diffraction analysis, and the resulting electron density profile of long-chain SM bilayers, suggest that at least 20:0-SM, 22:0-SM and 24:0-SM species show interdigitation [33]. Similar data on interdigitation were recently presented in a molecular dynamics simulation study on long-chain SMs [34].

Gel-phase destabilization was not clearly reported by DPH when its steady-state anisotropy was measured in SM bilayers (Fig. 1). This may relate to the observations that DPH anisotropy is not very responsive to changes in the packing properties of the gel phase, which is illustrated by the fact that increasing the cholesterol content in 16:0-SM bilayers up to 30 mol% did not significantly change DPH anisotropy in the gel/liquid ordered phase [22]. It is likely that both the gel phase and the liquid-

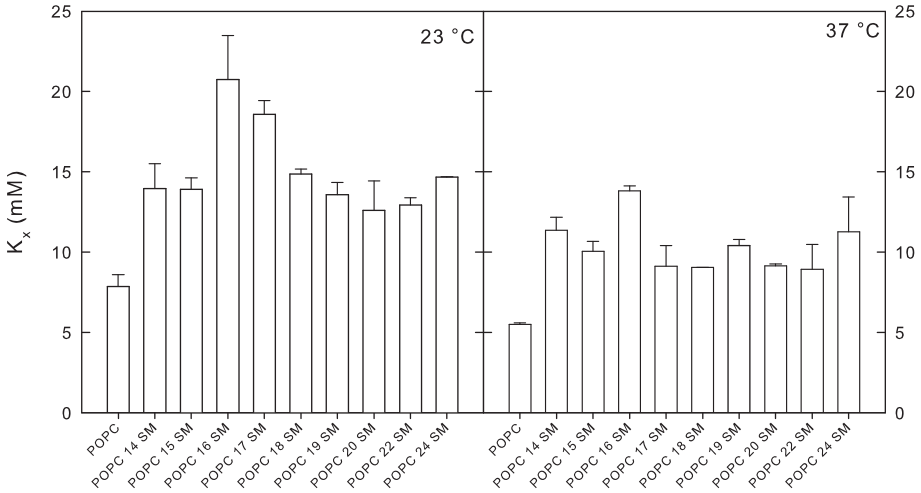

Fig. 5. Partitioning of CTL into unilamellar bilayer vesicles containing either POPC or POPC:SM analogs (4:1 molar ratio) at 23 °C (left panel), or 37 °C (right panel). CTL partitioning was determined as described in the experimental section and each value is the average from 3 to 4 separate experiments ± S.E.M.

ordered phase restrict the dynamics of DPH, and thus give high anisotropy values. In the fluid state, the DPH measured anisotropies were rather similar (<r> 0.13 ± 0.01) at a temperature of $T_m + 10$ °C (Fig. 1) for the different acyl chain analogs of SM. Atomistic simulation data, on the other hand, suggest that the calculated deuterium order parameter for the distal carbons in each SM chain length analog did decrease with increasing molecular mismatch. During steady-state DPH anisotropy measurements average anisotropy values are observed from DPH that possibly exist in different microenvironments. It is quite likely that the mismatch-induced disordering effect on the terminal carbons is sensed by a fraction of the DPH molecules, but the information is lost when the information from all DPH molecules are averaged during measurement.

The formation of sterol-enriched ordered domains in fluid POPC bilayers by the saturated chain-length SM analogs was measured from the quenching susceptibility of CTL. All SM analogs were able to form ordered domains in which a fraction of CTL was protected against quenching by 7SLPC in the fluid phase (Fig. 3). Some of the quenching protection was lost when the bilayers were exposed to higher temperatures, which is known to lead to fluidization of the ordered domains [17]. The finding that all SM analogs formed CTL-enriched domains strongly suggest that sterols (including cholesterol) are able to associate even with long-chain SMs in bilayers, despite the hydrophobic mismatch between the sterol molecules and the long acyl chains. A previous monolayer study has shown that cholesterol is able to interact with mismatched SM analogs [18]. However, that study was performed on binary monolayers containing equimolar amounts of cholesterol and SM analog, and in which the molecules were "forced" to interact with each other. The present study shows that even in a ternary bilayer, where CTL can choose to interact with either POPC or the SM analog, the sterol appears to prefer interaction with mismatched SM analogs over a fluid PC. However, CTL did not appear to have the same preference for all SM analogs, since the $\Delta F/F_0$ value was maximal with 16:0-SM and decreased with shorter or longer SM species (Fig. 4). The value for the $\Delta F/F_0$ is influenced by the amount of CTL in the domain in which CTL is protected from quenching. The $\Delta F/F_0$ could also be affected by the solubility of the quencher (7SLPC) in the ordered domains. It is, however, hard to envision an increased solubility of 7SLPC in SMs with increasing chain lengths, because of the bulky nature of the doxyl group in 7SLPC, and the gel-like nature of saturated long-chain SMs. Therefore the more plausible interpretation of the $\Delta F/F_0$ is that it reflects the amount of CTL (and cholesterol) in the ordered domain.

To better define the affinity of CTL to bilayers containing saturated long-chain analogs of SM, a partition assay was used to give the mol fraction partition coefficient of CTL [36,37]. Partitioning data for CTL clearly show that the highest affinity was for 16:0-SM-containing bilayers, and the affinity decreased for bilayers having shorter or longer-chain SM analogs. The partition data at 23 °C (Fig. 5 left panel) very closely mirror the $\Delta F/F_0$ data obtained from CTL quenching (Fig. 4), showing that two different methods give almost identical information about the preference of CTL interaction with SM. We thus can show for the first time that CTL (and therefore cholesterol) prefers 16:0-SM over other saturated SM analogs. This observation is consistent with previous DSC data showing that when cholesterol's interaction with symmetric PCs was determined, the best hydrophobic match for cholesterol was the C17 PC [29]. Androsterol, which lacks the isooctyl side chain of cholesterol, also has a best match when interacting with chain-length analogs of PCs in monolayer membranes [30]. Androsterol's best match was C14 PC, when interaction was determined from the desorption rate of androsterol from monolayers to cyclodextrins in the subphase. Obviously, cholesterol (or CTL), and androsterol must have different "best matches" because of their different hydrophobic lengths, but the principle that hydrophobic match affects molecular interaction appears to be valid and is supported by several studies [29,30,51,52].

In conclusion, we have demonstrated that CTL (and thus cholesterol) is able to interact with saturated SMs to form ordered domains, even when hydrophobic mismatch is present among the SMs. This is apparently not the case for mismatched phosphatidylcholines, at least not in monolayers [18]. However, of all saturated SM analogs, CTL (and cholesterol) appeared to favor interaction with 16:0-SM. This SM species apparently had the best hydrophobic match for CTL/cholesterol among all SM analogs tested. These results are important, since they show that although cholesterol may prefer to interact with 16:0-SM, it is likely to also interact favorably with all saturated SM species present in cell membranes and within lipid rafts, in which both SMs and cholesterol are enriched.

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