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ORIGINAL PAPER

The effects of *N*-acyl chain methylations on ceramide molecular properties in bilayer membranes

Terhi Maula · Bakarne Urzelai · J. Peter Slotte

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Abstract Long-chain saturated ceramides possess the ability to form gel domains in fluid bilayer membranes. Such domains may also contain sphingomyelin, but generally exclude cholesterol. We studied the effect of *N*-acyl chain methylations on the ability of ceramide to form ceramide- and sphingomyelin-containing gel domains that displace sterol. Fluorescence quenching of probes displaying different lateral partitioning in heterogeneous lipid bilayers showed that the methyl branches induced position-dependent changes in the lateral distribution of the ceramides. Distally monomethylated ceramides interacted with sphingomyelin and displaced sterol, whereas proximally monomethylated and polymethylated ceramides appeared to be located outside of sterol/sphingomyelin-enriched domains. The branched ceramides also markedly reduced the bilayer affinity for sterol as determined from the equilibrium partitioning of sterol between lipid vesicles and cyclodextrin. Altogether, alterations in intermolecular interactions induced by the methyl branches markedly affected the molecular properties of ceramide in artificial bilayers.

Keywords Ceramide gel phase · Sterol displacement · Fluorescence quenching · Sterol partitioning · *Trans*-parinaric acid · Cholestatrienol

T. Maula (✉) · B. Urzelai · J. Peter Slotte
Biochemistry, Department of Biosciences,
Åbo Akademi University, Tykistökatu 6 A,
20520 Turku, Finland
e-mail: terhi.maula@abo.fi

Present Address:
B. Urzelai
Department of Biochemistry and Molecular Biology,
University of the Basque Country, 48080 Bilbao, Spain

Abbreviations

(P)SM	(Palmitoyl-)sphingomyelin
PCer	Palmitoyl-ceramide
10MeCer	*N*-10-methyl-hexadecanoyl-ceramide
15MeCer	*N*-15-methyl-heptadecanoyl-ceramide
16MeCer	*N*-16-methyl-heptadecanoyl-ceramide
PhytCer	*N*-3,7,11,15-methyl-hexadecanoyl-ceramide
tPA	*Trans*-parinaric acid
CTL	Cholestatrienol
CHL	Cholesterol

Introduction

Ceramides constitute only a minor subclass of freely existing sphingolipids in cells, but are still associated with important cellular signaling pathways (Hannun and Luberto 2000; Perry and Hannun 1998). Some of the ceramide-mediated signaling has been suggested to be related to structural changes induced by ceramides in bilayer membranes. Indeed, contributions of ceramides to membrane structure and thermodynamic behavior have been carefully examined in artificial bilayers, where long-chain ceramides stabilize and increase the order of fluid membranes, either by laterally segregating to ceramide-enriched gel phase domains (Silva et al. 2006) or by interacting with sphingomyelin (SM) to form ceramide/SM gel phases (Alanko et al. 2005; Massey 2001). The ultimate function of ceramides in cellular membranes is still unclear, but it is proposed that, upon formation, ceramide-enriched platforms aid in transmitting and amplifying trans-membrane signals (Stancevic and Kolesnick 2010).

Because of competition over interactions with SM, ceramides and cholesterol affect each other's lateral distribution in membranes. Ceramides have been shown to displace

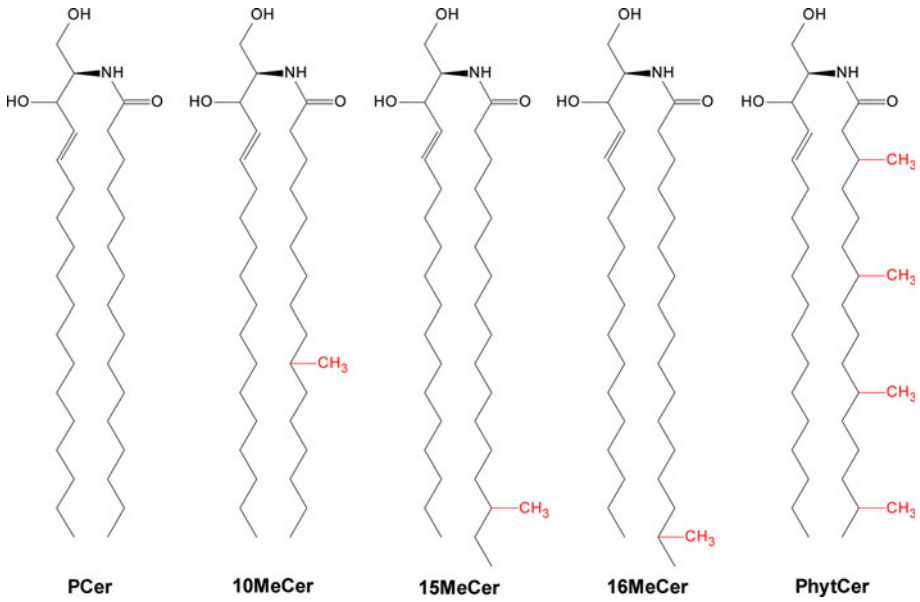

Fig. 1 Structures of the ceramides used in this study

cholesterol from caveolin-rich membrane rafts enriched in SM and cholesterol (Yu et al. 2005), and from the vicinity of SM in artificial bilayers (Alanko et al. 2005; Megha and London 2004; Sot et al. 2008). High concentrations of cholesterol may, in turn, solubilize ceramide-enriched domains (Castro et al. 2009) or even prevent the lateral cosegregation of ceramide and SM (Busto et al. 2010). The favorable interactions between ceramide and SM originate from the close interactions between their acyl chains. Consequently, modifications of ceramide structure ought to affect its ability to interact with SM and to regulate the lateral distribution of cholesterol in membranes. Interestingly, ceramides that have been chemically modified at the long-chain base near to the head group region (C1–C7) induce similar thermal stabilization and displacement of sterol from SM-enriched domains as unmodified ceramides (Megha et al. 2007). However, the ability of ceramides to form ceramide/SM-rich domains that exclude sterol is significantly altered for ceramides with varying acyl chain lengths (Nybond et al. 2005; Nyholm et al. 2010). Considering the altered membrane behavior of structurally diverse ceramides, it seems that the amide-linked acyl chain is one of the major determinants of ceramide's interactions with other lipids such as SM.

Interestingly, some naturally occurring ceramides contain branched acyl chains. The branches most often appear in the long-chain base, but also branched amide-linked acyl chains have been found in natural ceramides. For example, as much as 35% of the acyl chains in the ceramide aminoethylphosphonates of the sea anemone *Metridium senile* have been found to contain *iso* and *anteiso* methyl branches in acyl chains of varying length (Karlsson and Samuelsson 1974). Also other aquatic animals and microorganisms have ceramides with methyl-branched acyl chains (Tanaka et al. 1998; Tian et al. 2009), and *iso*-branched ceramides have also been found in *Sphingobacterium spiritivorum* (Yano et al. 1983). In mammals, methyl-branched ceramide acyl chains have been found in the guinea pig Harderian gland (Yasugi et al. 1987, 1991) and in human *vernix caseosa*, the waxy coating of a fetus or a newborn baby (Oku et al. 2000). Ceramides with methyl branches in the middle region (carbon 8 or 10) of the long-chain base have also been found in mammals (Yasugi et al. 1988). Characteristic for the different methyl-branched ceramides is that many of them also contain additional hydroxyl groups and double bonds, and some are polymethylated, having several methyl branches in the amide-linked acyl chain or long-chain base, or in both.

To study the molecular properties of acyl chain methylated ceramides in bilayer membranes, we synthesized four different ceramide analogs, the singly methylated *N*-10-methyl-hexadecanoyl-ceramide (10MeCer), *N*-15-methyl-heptadecanoyl-ceramide (15MeCer), and *N*-16-methyl-heptadecanoyl-ceramide (16MeCer), and the polymethylated *N*-3,7,11,15-methyl-hexadecanoyl-ceramide (PhytCer) with phytanic acid as the acyl chain (Fig. 1). The ability of these ceramides to interact with SM to form ceramide/SM-domains that exclude sterol was then determined from the quenching susceptibility of fluorescent probes displaying different lateral partitioning in laterally heterogeneous lipid bilayers. In addition, the overall affinity of sterol for bilayers containing the ceramides was determined.

Experimental approach

Material

D-*erythro*-*N*-palmitoyl-sphingomyelin (PSM) was purified from egg yolk sphingomyelin (Avanti Polar Lipids) as

previously described in Maula et al. (2009). 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was from Avanti Polar Lipids, and cholesterol and methyl-β-cyclodextrin (mβ-Cyd) from Sigma Chemicals. D-*erythro*-*N*-palmitoyl-ceramide (PCer), 10-methyl-hexadecanoic acid, 15-methyl-heptadecanoic acid (*anteiso*), 16-methyl-heptadecanoid acid (*iso*) and phytanic acid (3,7,11,15-methyl-hexadecanoic acid) were from Larodan Fine Chemicals.

The ceramide analogs, *N*-10-methyl-hexadecanoyl-ceramide (10MeCer), *N*-15-methyl-heptadecanoyl-ceramide (15MeCer), *N*-16-methyl-heptadecanoyl-ceramide (16MeCer) and *N*-3,7,11,15-methyl-hexadecanoyl-ceramide (PhytCer), were synthesized from the methylated fatty acids and D-*erythro*-sphingosine (Sigma Chemicals) according to Cohen et al. (1984). The ceramide products were purified by reverse-phase HPLC with methanol:acetonitrile (70:30 by volume for 15MeCer, 16MeCer and PhytCer, or 50:50 for 10MeCer) as the eluent (UV detection 203 nm), and positively verified with the Bruker Daltonics Ion Trap-ESI-MS. All lipid stock solutions were prepared in hexane:isopropanol (3:2 by volume), stored at −20°C and warmed to ambient temperature before use.

Trans-parinaric acid (tPA) was from Cayman Chemical Co. Cholesta-5,7,9(11)-trien-3-beta-ol (CTL) was synthesized and purified as described in Fischer et al. (1984). 1-Palmitoyl-2-stearoyl-(7-doxyl)-*sn*-glycero-3-phosphocholine (7SLPC) was synthesized from (7-doxyl)-stearic acid (TCI Europe) and 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (Avanti Polar Lipids) according to Bittman (1993). tPA, CTL and 7SLPC were stored dry under argon in the dark at −87°C until dissolved in methanol, ethanol or hexane:isopropanol (3:2 by volume), respectively, prior to use. Water for sample preparation was purified by reverse osmosis and the Millipore UF Plus water-purification system.

Vesicle preparation

Multilamellar vesicles (50 μM) for fluorescence quenching experiments were prepared by drying the mixtures of lipids, probes and quencher under a stream of nitrogen and saturating the lipid films with argon. The samples were stored at −20°C until hydrated one at a time for 15 min with pre-heated, argon-purged water at 65°C. Multilamellar vesicles were formed at 65°C by sonicating the CTL-containing samples with a probe sonifier (Branson Ultrasonics) for 2 min, and the tPA-containing samples with a Branson bath sonicator for 5 min. For each quenching curve two samples were prepared, the quenched F-sample and the unquenched F_0-sample. The total amount of lipids in all samples was 100 nmol. Both the F- and the F_0-samples also contained an additional 1 mol % of the fluorophore (tPA or CTL). All F-samples also contained 30 mol % of the quencher, 7SLPC, which replaced an equal amount of POPC in the F-samples. This quenching method is based on the different lateral partitioning of the fluorophores and the quencher in a mixed bilayer. While the fluorophores are located in phase-separated domains of increased molecular order (tPA in highly ordered SM-containing domains and CTL in sterol-enriched domains), the quencher strongly partitions into fluid phases. At low temperatures this results in high F/F_0-values as the phase separation occurring in the bilayer is shielding the fluorophores from collision-induced quenching by 7SLPC. At higher temperatures the phase separation eventually will disappear, allowing closer contact between the fluorophores and the quencher, resulting in strong quenching of the fluorescence signal and a decrease in the F/F_0-value.

Large unilamellar vesicles for the sterol partitioning assay were prepared as described in Nyholm et al. (2010). Shortly, lipids were mixed at the desired molar ratios, including 2 mol% CTL, and dried under a stream of nitrogen. The lipid films were then hydrated for 30 min at 65° and vortexed briefly to form multilamellar vesicles. The multilamellar vesicles were then extruded through a membrane with 200-nmol pores to yield unilamellar vesicles. The unilamellar vesicles were then portioned into a series of samples with increasing concentrations of mβ-Cyd and diluted to a final concentration of 40 μM lipids and 0–1 mM mβ-Cyd. In quaternary mixtures of POPC/PSM/CHL/XCer (60/20/20/15 molar ratio), the ceramides were added to the initial lipid mixtures to yield 15 mol% of ceramide in vesicles with a final lipid concentration of ~52 μM. Before partitioning measurements, the samples were incubated to equilibrium over night at room temperature or 2 h at 37°C.

Fluorescence quenching measurements

Fluorescence quenching of tPA (excitation 305 nm, emission 405 nm) and CTL (excitation 324 nm, emission 390 nm) by 7SLPC was measured on a QuantaMaster-1-spectrofluorometer (Photon Technology International) as a function of increasing temperature. The fluorescence signal of the fluorophores was scanned continuously under constant stirring while heating the samples at a rate of 5°C/min controlled by a Peltier element. Fluorescence quenching, reported as the F/F_0-ratio (Bjorkqvist et al. 2005), was calculated with the PTI FeliX32-software.

Sterol partitioning assay

The equilibrium partitioning of CTL between unilamellar vesicles and mβ-Cyd was determined by measuring the anisotropy of CTL (excitation 324 nm, emission 390 nm) in a series of samples with increasing concentrations of

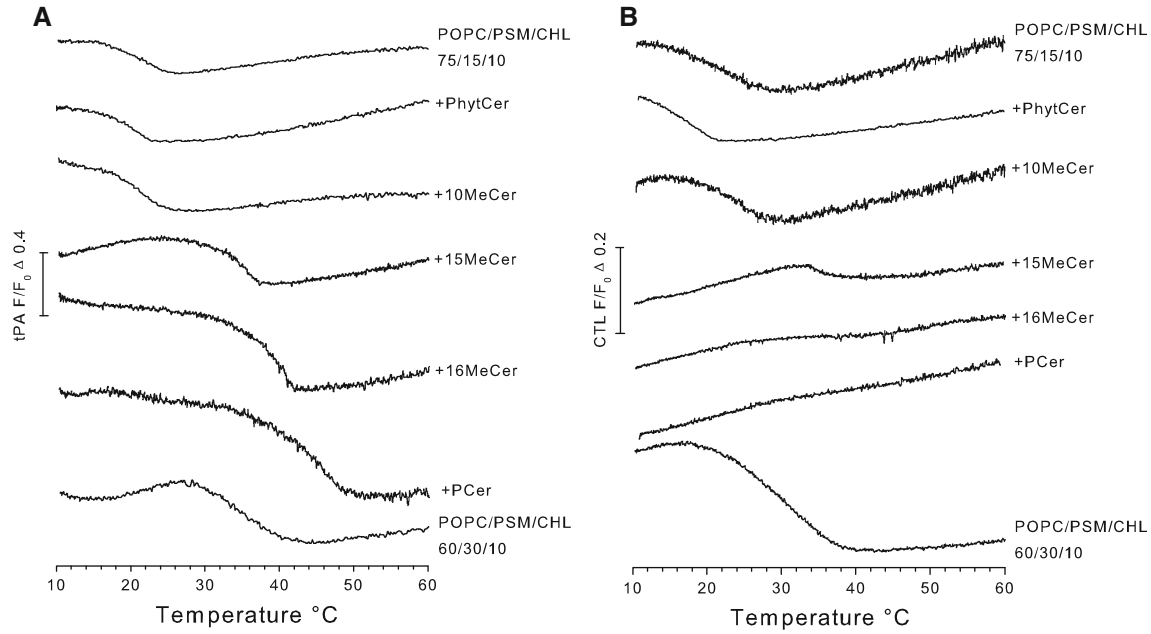

Fig. 2 Fluorescence quenching of **a** tPA and **b** CTL in bilayers containing acyl chain methylated ceramides. Fluorescence quenching was determined as a function of temperature in laterally heterogeneous bilayers composed of POPC/PSM/CHL (60/30/10 or 75/15/10) and POPC/PSM/XCer/CHL (60/15/15/10). Representatives of at least two independently reproduced *identical curves* are shown

mβ-Cyd. Anisotropy was measured for 30 s on a Quanta-Master-1-spectrofluorometer, and the temperature controlled by a Peltier element under constant stirring. CTL anisotropies were then converted to molar fraction partition coefficients as previously described in Nystrom et al. (2010).

Results and discussion

Acyl chain methylated ceramides displayed differences in the ability to form gel phases that exclude sterol

To determine the ability of the methylated ceramides to interact with PSM to form gel phase domains that displace sterol, the fluorescence quenching of tPA (Fig. 2a) and CTL (Fig. 2b) was determined as a function of increasing temperature in mixed bilayers. Since tPA partitions into both sterol/PSM and ceramide/PSM domains, whereas CTL only partitions into sterol-enriched domains and not into ceramide-enriched domains (Bjorkqvist et al. 2005), comparison of the fluorescence quenching behavior of tPA and CTL was used to reveal whether the bilayers contained ceramide/PSM domains that excluded sterol. In the absence of ceramide, in bilayers composed of POPC/PSM/CHL (60/30/10 mol %), sterol/PSM domains were observed with both tPA and CTL. The melting temperature of these domains was determined from the quenching curves as the approximate temperature at the end point of the decreasing F/F_0-value, after which this ratio remained more or less unchanged, indicating that the sterol/PSM domains had melted. The slightly higher end-melting temperature of the sterol/PSM domains reported by tPA (42°C) than by CTL (40°C) was probably related to the stabilizing effect that tPA has on gel phases (Ben et al. 1987; Sklar et al. 1977).

Replacing half of the PSM in the bilayers with PCer increased the end-melting temperature of the gel phase up to 50°C as reported by tPA, indicating that PCer associated within and stabilized the PSM-rich domains. CTL again did not report any detectable domain melting for the PCer-containing bilayers, indicating that CTL was not able to partition into the PCer/PSM domains that were formed. Instead, CTL was displaced to the fluid bulk membrane, where it became quenched by 7SLPC. The gentle increase in the F/F_0-ratio of the CTL curve in the presence of PCer was due to increased background noise at low fluorescence signal intensities (originating from rapid quenching of the CTL-signal in the F-sample). For 16MeCer- and 15MeCer-containing bilayers tPA reported a gel phase end-melting temperature of 42 and 38°C, respectively, while CTL-quenching showed that also 16MeCer and 15MeCer displaced sterol from the PSM-rich domains. The lower end-melting temperature of the ceramide/PSM-domains formed by 16MeCer and 15MeCer compared to PCer indicate that these analogs were able to associate within, but did not thermally stabilize the PSM domains. This suggests that structural constraints induced by the methylations resulted in less tight acyl chain packing between the ceramide

analogs and PSM. Also, as some enrichment of CTL could still be observed in the presence of 16MeCer and 15MeCer, reporting approximately the same end-melting temperatures as tPA, the displacement of sterol was concluded to be less efficient than with PCer.

Interestingly, compared to the ceramide-free vesicles (POPC/PSM/CHL; 60/30/10), 10MeCer and PhytCer appeared to markedly decrease the melting temperature of the PSM-rich domains as reported by tPA. Also, no displacement of sterol could be observed since also CTL reported domain melting for those bilayers. Since no sterol displacement was observed by these analogs, and tPA and CTL reported almost equal end-melting temperatures for the domains in these bilayers, we also analyzed bilayers composed of POPC/PSM/CHL in 75/15/10 molar composition. Interestingly, the melting curves of 10MeCer and PhytCer were very similar to that of the 75/15/10-control vesicles, suggesting that 10MeCer and PhytCer, instead of partitioning within the PSM-rich domains, remained outside the domains in the fluid POPC-phase or perhaps at the domain boundaries. Therefore, what was observed in the presence of 10MeCer and PhytCer was actually the melting of sterol/PSM-rich domains, like those present in the 75/15/10-composition. However, in vesicles that contained PhytCer, the end-melting temperature of the sterol/PSM-enriched domains was slightly decreased compared to the ceramide-free bilayers (75/15/10-composition). Taking into consideration the remarkable effect that the four methyl groups must have on the membrane properties of PhytCer, this ceramide probably decreased the melting temperature of the sterol/PSM domains by disordering the whole bilayer.

The results from the quenching experiments are consistent with previous observations on the effects of acyl chain methylation on the molecular space requirement and lateral head group interactions of ceramide in monolayer studies (Lofgren and Pascher 1977). This study showed that a methyl branch at carbon 11 of an eicosanoyl chain increased the space requirement of ceramide, resulting in steric hindrance against close acyl chain packing and loss of polar head group interactions. The same study also showed that a *cis*-double bond at carbon 9 of an octadecanoyl chain induced similar effects as the methyl branch. However, for a ceramide with a *cis*-double bond at carbon 15 in nervonic acid coupled to sphingosine backbone, the molecular area in monolayers remained unchanged compared to the corresponding ceramide with a fully saturated acyl chain. In line with these and our results, the position-dependent effects of methyl branches on ceramide bilayer behavior seem to closely resemble the position-dependent effects of *cis*-double bonds.

Our results also agree well with experiments made with methylated SMs (Jaikishan et al. 2010), showing that, like 16MeCer and 15MeCer, also 16MeSM and 15MeSM were able to form gel phases and ordered domains, even if their packing properties were slightly affected by the distal methyl branches. In the same study, 10MeSM and PhytSM were shown to fail in the formation of gel phases or ordered domains, correlating well with our observations about the fluid nature of 10MeCer and PhytCer.

Sterol affinity for bilayers was reduced in the presence of acyl chain methylated ceramides

Since the quenching results indicated that the methyl groups had position-dependent effects on the lateral distribution of the ceramides in the bilayers, we also examined how the presence of methylated ceramides (either within PSM-rich domains or in the fluid phase) affected the overall affinity of sterol for bilayers (Fig. 3). At 23°C, addition of 25 mol % of PSM into pure POPC bilayers increased the molar fraction partition coefficient (K_x) of sterol from 8 to 25 mM, which is in accordance with the previous observation that the K_x of sterol for DOPC bilayers increases with increasing amounts of PSM in the bilayers (Nyholm et al. 2010). Compared to the POPC/PSM bilayers, CTL reported a markedly decreased affinity for POPC/PSM/PCer bilayers (60/20/20-composition, K_x 12 mM). This reduction in K_x originates from the sterol-displacing effect of PCer, driving CTL out of the PSM-enriched domains to the more fluid POPC phase from which it is more easily accessible to mβ-Cyd. For POPC/PSM/CHL bilayers (60/20/20 mol %), the affinity was again significantly increased, with K_x having

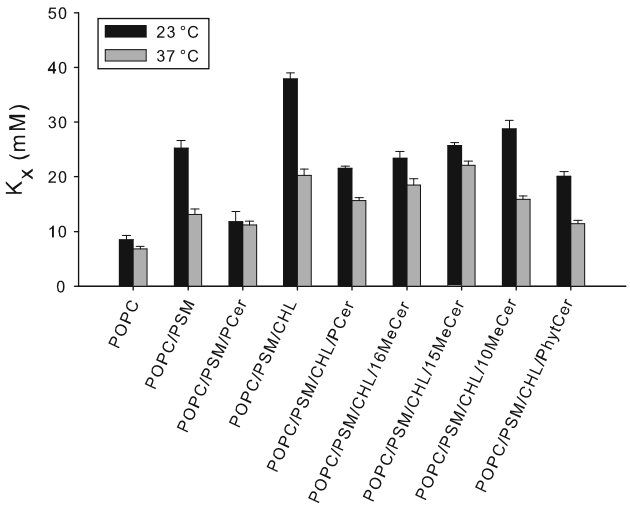

Fig. 3 CTL-partitioning at 23°C (*black bars*) and 37°C (*gray bars*) in the presence of acyl chain methylated ceramides. The bilayer affinity of sterol was determined for unilamellar vesicles composed of pure POPC, POPC/PSM (60/20), POPC/PSM/PCer (60/20/20), POPC/PSM/CHL (60/20/20) or POPC/PSM/CHL/XCer (60/20/20/15). Each value is the average of at least three independently repeated experiments ±SD

a value of 38 mM. These observations clearly indicate that the CTL-partitioning assay responded well to changes in the composition of the bilayers.

When 15 mol % of PCer was added to the POPC/PSM/CHL bilayers (yielding a POPC/PSM/CHL/PCer 60/20/20/15-composition), the affinity of sterol for the bilayers was reduced from 38 to 22 mM at 23°C. Similarly, addition of 15 mol % of 16MeCer or 15MeCer to the bilayers reduced the K_x to 23 and 26 mM, respectively, indicating that both 16MeCer and 15MeCer were able to interact with PSM to displace sterol. However, the effects of 16MeCer and 15MeCer on sterol affinity were not as strong as with PCer, which was consistent with the quenching data.

10MeCer, with the K_x of 29 mM, was least effective in reducing sterol affinity, whereas PhytCer proved to be the most effective, displaying a partition coefficient of 20 mM at 23°C. The K_x of the 10MeCer-containing samples probably reflected sterol affinity for the sterol/PSM-rich domains, but was lower than in the ceramide-free bilayers because of destabilization of the fluid phase or the domain boundaries by 10MeCer, causing increased desorption of sterol to mβ-Cyd. PhytCer presumably induced some considerable defects in the bilayer structure, resulting in significant destabilization of the entire bilayer, decreasing sterol affinity both for the fluid phase and for the sterol/PSM-rich domains.

Sterol partitioning was generally lower at 37°C than at 23°C, but followed more or less the same pattern at both temperatures. The relative differences between different samples were also smaller at 37°C than at 23°C, suggesting that a larger fraction of the bilayers was in a fluid state at 37°C, probably because of the smaller size and reduced stability of the ordered domains. For pure POPC bilayers and for the POPC/PSM/PCer bilayers, sterol affinity remained at the same level at 23 and 37°C. Pure POPC bilayers are at a totally fluid state already at 23°C, and therefore no difference in K_x was observed by increasing the temperature. For POPC/PSM/PCer bilayers, the K_x remained unchanged because in these bilayers CTL was probably fully displaced from the PCer/PSM gel phase domains into the fluid POPC phase for which the fluidity was not significantly changed at the two different temperatures. An interesting observation was the low K_x represented for the 10MeCer-containing bilayers at 37°C, a behavior deviating from the pattern observed for the ceramide analogs at 23°C. However, this could be a consequence of 10MeCer destabilizing the bilayer structure in the fluid phase even more at 37°C, resulting in a more pronounced effect on the sterol/PSM-rich domains than at 23°C.

Conclusions

These results clearly demonstrated that methyl branches in ceramide acyl chain affected, in terms of SM and sterol interactions, the membrane properties of ceramides in a position-dependent manner. Distal monomethylations interfered less with acyl chain packing and molecular interactions than proximal monomethylations or polymethylations. The acyl chain methylated ceramides also markedly reduced the bilayer affinity of sterol. This study shows that methyl branches in the ceramide acyl chain induce disorder and interference with molecular packing, which together with previous observations (Nybond et al. 2005; Nyholm et al. 2010) augment the important role of the *N*-acyl chain structure in determination of ceramide membrane behavior. In a similar way, also methyl branches in the long-chain base would be expected to affect the membrane behavior of ceramides. In biological membranes methyl branches could serve similar functions as *cis* double bonds, conferring the ceramides a more fluid nature, but by a less reactive chemical group.

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References

Alanko SM, Halling KK, Maunula S, Slotte JP, Ramstedt B (2005) Displacement of sterols from sterol/sphingomyelin domains in fluid bilayer membranes by competing molecules. Biochim Biophys Acta 1715:111–121

Ben YV, Menashe M, Biltonen RL, Johnson ML, Barenholz Y (1987) Interaction of trans-parinaric acid with phosphatidylcholine bilayers: comparison with the effect of other fluorophores. Biochim Biophys Acta 904:117–124

Bittman R (1993) Chemical preparation of glycerolipids: a review of recent syntheses in Phospholipids Handbook. In: Cevec G (ed) Marcel Dekker, Inc., pp 154–155

Bjorkqvist YJ, Nyholm TK, Slotte JP, Ramstedt B (2005) Domain formation and stability in complex lipid bilayers as reported by cholestatrienol. Biophys J 88:4054–4063

Busto JV, Sot J, Requejo-Isidro J, Goni FM, Alonso A (2010) Cholesterol displaces palmitoylceramide from its tight packing with palmitoylsphingomyelin in the absence of a liquid-disordered phase. Biophys J 99:1119–1128

Castro BM, Silva LC, Fedorov A, de Almeida RF, Prieto M (2009) Cholesterol-rich fluid membranes solubilize ceramide domains: implications for the structure and dynamics of mammalian intracellular and plasma membranes. J Biol Chem 284:22978–22987

Cohen R, Barenholz Y, Gatt S, Dagan A (1984) Preparation and characterization of well defined D-erythro sphingomyelins. Chem Phys Lipids 35:371–384

Fischer RT, Stephenson FA, Shafiee A, Schroeder F (1984) delta 5, 7, 9(11)-Cholestatrien-3 beta-ol: a fluorescent cholesterol analogue. Chem Phys Lipids 36:1–14

Hannun YA, Luberto C (2000) Ceramide in the eukaryotic stress response. Trends Cell Biol 10:73–80

Jaikishan S, Bjorkbom A, Slotte JP (2010) Sphingomyelin analogs with branched N-acyl chains: the position of branching dramati-

cally affects acyl chain order and sterol interactions in bilayer membranes. Biochim Biophys Acta 1798:1987–1994

Karlsson KA, Samuelsson BE (1974) The structure of ceramide aminoethylphosphonate from the sea anemone, Metridium senile. Biochim Biophys Acta 337:204–213

Lofgren H, Pascher I (1977) Molecular arrangements of sphingolipids. The monolayer behaviour of ceramides. Chem Phys Lipids 20:273–284

Massey JB (2001) Interaction of ceramides with phosphatidylcholine, sphingomyelin and sphingomyelin/cholesterol bilayers. Biochim Biophys Acta 1510:167–184

Maula T, Westerlund B, Slotte JP (2009) Differential ability of cholesterol-enriched and gel phase domains to resist benzyl alcohol-induced fluidization in multilamellar lipid vesicles. Biochim Biophys Acta 1788:2454–2461

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

Megha, Sawatzki P, Kolter T, Bittman R, London E (2007) Effect of ceramide N-acyl chain and polar headgroup structure on the properties of ordered lipid domains (lipid rafts). Biochim Biophys Acta 1768:2205–2212

Nybond S, Bjorkqvist YJ, Ramstedt B, Slotte JP (2005) Acyl chain length affects ceramide action on sterol/sphingomyelin-rich domains. Biochim Biophys Acta 1718:61–66

Nyholm TK, Grandell PM, Westerlund B, Slotte JP (2010) Sterol affinity for bilayer membranes is affected by their ceramide content and the ceramide chain length. Biochim Biophys Acta 1798: 1008–1013

Nystrom JH, Lonnfors M, Nyholm TK (2010) Transmembrane peptides influence the affinity of sterols for phospholipid bilayers. Biophys J 99:526–533

Oku H, Mimura K, Tokitsu Y, Onaga K, Iwasaki H, Chinen I (2000) Biased distribution of the branched-chain fatty acids in ceramides of vernix caseosa. Lipids 35:373–381

Perry DK, Hannun YA (1998) The role of ceramide in cell signaling. Biochim Biophys Acta 1436:233–243

Silva L, de Almeida RF, Fedorov A, Matos AP, Prieto M (2006) Ceramide-platform formation and -induced biophysical changes in a fluid phospholipid membrane. Mol Membr Biol 23:137–148

Sklar LA, Hudson BS, Simoni RD (1977) Conjugated polyene fatty acids as fluorescent probes: synthetic phospholipid membrane studies. Biochemistry 16:819–828

Sot J, Ibarguren M, Busto JV, Montes LR, Goni FM, Alonso A (2008) Cholesterol displacement by ceramide in sphingomyelin-containing liquid-ordered domains, and generation of gel regions in giant lipidic vesicles. FEBS Lett 582:3230–3236

Stancevic B, Kolesnick R (2010) Ceramide-rich platforms in transmembrane signaling. FEBS Lett 584:1728–1740

Tanaka II, Matsuoka S, Murata M, Tachibana K (1998) A new ceramide with a novel branched-chain fatty acid isolated from the epiphytic dinoflagellate coolia monotis. J Nat Prod 61:685–688

Tian XR, Tang HF, Li YS, Lin HW, Ma N, Zhang W, Yao MN (2009) Ceramides and cerebrosides from the marine bryozoan Bugula neritina inhabiting South China Sea. J Asian Nat Prod Res 11:1005–1012

Yano I, Imaizumi S, Tomiyasu I, Yabuuchi E (1983) Separation and analysis of free ceramides containing 2-hydroxy fatty acids in *Sphingobacterium* species. FEMS Microbiol Lett 20:449–453

Yasugi E, Kasama T, Seyama Y (1987) Branched long chain bases in cerebrosides of the guinea pig Harderian gland. J Biochem 102:1477–1482

Yasugi E, Kasama T, Seyama Y (1988) Identification of 10-methylsphinganine in cerebrosides of the guinea pig harderian gland. J Biochem 103:889–893

Yasugi E, Kasama T, Seyama Y (1991) Composition of long chain bases in ceramide of the guinea pig Harderian gland. J Biochem 110:202–206

Yu C, Alterman M, Dobrowsky RT (2005) Ceramide displaces cholesterol from lipid rafts and decreases the association of the cholesterol binding protein caveolin-1. J Lipid Res 46:1678–1691