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Molecular features of phospholipids that affect glycolipid transfer protein-mediated galactosylceramide transfer between vesicles

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Abstract

The glycolipid transfer protein (GLTP)-mediated movement of galactosylceramide from model membrane donor vesicles to acceptor vesicles is sensitive to the membrane environment surrounding the glycolipid. GLTP can catalyze the transfer of a fluorescently labeled GSL, anthrylvinyl-galactosylceramide (AV-GalCer), from vesicles composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine and dipalmitoylphosphatidylcholine matrices, but not from vesicles prepared from *N*-palmitoylsphingomyelin, regardless of the cholesterol content of the vesicles. In this study, we have examined the structural features of sphingomyelin (SM) that are responsible for its inhibition of the rate of GLTP-catalyzed transfer of AV-GalCer. The rate of glycolipid transfer was enhanced when the *N*-palmitoyl chain of SM was replaced with an *N*-oleoyl chain. Analogs of *N*-palmitoyl-SM in which the 4,5-double bond of the long-chain base is reduced or the 3-hydroxy group is removed did not inhibit GLTP-catalyzed transfer of AV-GalCer. When the donor vesicles were prepared with phosphatidylcholines or ether-linked phosphatidylcholine analogs, the transfer rates of AV-GalCer increased with increasing degree of unsaturation. The rate of AV-GalCer transfer was strongly dependent on the unsaturation degree of the acyl and/or alkyl chains. For ester-linked PCs, the transfer rate increased in the order DPPC < POPC < DOPC, which have 0, 1, and 2 *cis* double bonds, respectively.

Keywords: Sphingomyelin; Phosphatidylcholine; Fluorescence; glycosphingolipid; Cholesterol; Glycolipid transfer protein; Sphingomyelin analog; Ether lipid

1. Introduction

The phospholipid sphingomyelin (*N*-acylsphingosine-1-phosphocholine, SM) bears a long chain fatty acid amide linked to the sphingoid base. The sphingoid base prevalent in most naturally occurring SMs possesses a 4,5-*trans* double bond and a 3-hydroxy group. SM displays unique properties compared with glycerophospholipids by carrying both hydrogen-bonding donor and hydrogen bond acceptor sites; phosphatidylcholine only has hydrogen bond acceptor sites. Glycosphingolipids (GSLs) differ from SM in that they carry one or more sugar residues in place of the zwitterionic phosphocholine headgroup. GSLs also have both hydrogen bond donor and acceptor properties and display high phase transition melting temperatures and tight membrane packing properties [1]. Sphingolipids, together with cholesterol, form

Abbreviations: SUV, small unilamellar vesicles; GLTP, glycolipid transfer protein; GSL, glycosphingolipids; PSM, *N*-palmitoyl-D-*erythro*-sphingosylphosphorylcholine; PDHSM, *N*-palmitoyl-D-*erythro*-dihydrosphingosylphosphoryl choline; OSM, *N*-oleoyl-D-*erythro*-sphingosylphosphorylcholine; ODHSM, *N*-oleoyl-D-*erythro*-dihydrosphingosylphosphorylcholine; 3-deoxy-PSM, 3-deoxy-*N*-palmitoylsphingosylphosphorylcholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; HPPC, 1-*O*-hexadecyl-2-(deoxy-*N*-palmitoylamido)-*sn*-glycero-3-phosphocholine; DHPC, 1,2-di-*O*-hexadecyl-*sn*-glycero-3-phosphocholine; di-oleyl-PC, 1,2-di-*O*-octadec-9(*Z*)-enyl-*sn*-glycero-3-phosphocholine; HOPC, 1-*O*-hexadecyl-2-oleoyl-*sn*-glycero-3-phosphocholine; AV-GalCer, *N*-[(11*E*)-12-(9-anthroyl)-11-dodecenoyl]-1-*O*-β-galactosylsphingosine; $DiOC_{16}$, 3,3′-dihexadecyloxacarbocyanine perchlorate

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stable lateral structures that are strengthened by both intra- and intermolecular hydrogen bonding networks [2].

SM is an abundant component of mammalian membranes. In addition to its important structural features, SM is a precursor of many lipid mediators that participate in diverse cell signaling processes, such as apoptosis and proliferation [3]. A SM variant that lacks the 4,5-*trans* double bond, referred to as dihydrosphingomyelin (DHSM), usually occurs in small quantities, but accounts for about 80% of the total SM of human lens membranes [4].

Glycolipid transfer proteins (GLTPs, 24 kDa, p*I* 9.0) have been identified in different cell and tissue types, including spleen [5], brain [6], and liver [7]. GLTPs catalyze the intermembrane transfer of various glycosphingolipids, including neutral glycosphingolipids and gangliosides in vitro [8,9], but its in vivo function remains elusive. Although the number of sugar residues is not critical for the transfer selectivity, a β-glycosidic bond between the sugar residue and the ceramide backbone is required [10]. Several characteristics of mammalian GLTPs suggest that these proteins are different from other known lipid transfer proteins. The mammalian GLTPs fold with a previously unknown two-layer all α-helical topology and has an adaptive ligand-binding site. The ligand-bound crystal structures of the bovine [11] and human [12] GLTPs have been solved. GLTP has sequence homology with two lipid-binding proteins: the HET-C2 protein of *Podospora anserina* (fungi) [13] and ACD11 of *Arabidopsis thaliana* (plant) [14]. GLTP action is proposed to involve formation of a complex between the protein and the glycolipid from the interface [15].

GLTP can catalyze the transfer of a fluorescently labeled GSL, *N*-[(11*E*)-12-(9-anthryl)-11-dodecenoyl]-1-*O*-β-galactosylsphingosine (AV-GalCer), from vesicles composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) matrices, but not from vesicles prepared from *N*-palmitoylsphingomyelin (PSM), regardless of the cholesterol content of the vesicles [16]. Here, we have used synthetic analogs of SM to analyze the structural features responsible for the inhibition of the protein-mediated glycosphingolipid transfer by PSM. We also analyzed the activity of GLTP in matrix lipids lacking the amide linkage, as in ester/ether-PC, diether-PC, and diester-PC, and an amide-linked/ether-linked PC.

2. Materials and methods

2.1. Materials

Cholesterol, DPPC, POPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-di-*O*-octadec-9(*Z*)-enyl-*sn*-glycero-3-phosphocholine (di-oleyl-PC), 1-*O*-hexadecyl-2-oleoyl-*sn*-glycero-3-phosphocholine (HOPC), 1,2-di-*O*-hexadecyl-*sn*-glycero-3-phosphocholine (DHPC), *N*-oleoyl-D-*erythro*-sphingomyelin (OSM), and egg SM were from Avanti Polar Lipids (Alabaster, AL, USA). Dihydro-SM and oleic acid were from Larodan Fine Chemicals (Mölndal, Sweden). 3,3′-Dihexadecyloxacarbocyanine perchlorate ($DiOC_{16}$) was from Invitrogen (Carlsbad, CA, USA), and dicyclohexylcarbodiimide, triethylamine, and palladium were from Fluka (Buchs, Switzerland). Butylated hydroxytoluene was from Sigma (St. Louis, MO, USA) and Triton X-100 from ICN Biomedicals (Aurora, OH, USA). *N*-Palmitoyl-D-*erythro*-SM (PSM) was purified from egg SM using reverse-phase HPLC (Discovery C18 column, 5 μm, 250 mm × 21.2 mm, with methanol as the solvent, and a flow rate of 9 ml/min). *N*-palmitoyl-D-*erythro*-dihydro-SM (PDHSM) was prepared from PSM by hydrogenation in ethanol using palladium as catalyst [17]. *N*-oleoyl-D-*erythro*-dihydro-SM (ODHSM) was synthesized from dihydrosphingosylphosphorylcholine and oleic acid as described recently [18], based on earlier work [19] and purified as described for PSM. 3-Deoxy-*N*-palmitoyl-SM (3-deoxy-SM) was synthesized as described previously [20], as were 1-*O*-alkyl-2-amido-phosphatidylcholine [21] and AV-GalCer [22]. The concentrations of the different phospholipids were determined by the Bartlett method [23]. The concentrations of the probes were determined gravimetrically (MT5, Mettler-Toledo, Columbus, OH, USA), which agreed with determinations based on the molar extinction coefficients. Recombinant bovine GLTP was expressed and purified as described earlier [24].

2.2. Preparation of phospholipid vesicles

The donor vesicles with the fluorescent probes AV-GalCer (1 mol%) and $DiOC_{16}$ (3 mol%) in a 96 mol% matrix of the respective phospholipids or binary mixtures of cholesterol with phospholipids (for structures, see Fig. 1) were prepared by probe sonication [25]. Briefly, the lipids were mixed with AV-GalCer and $DiOC_{16}$ from stock solutions in hexane/2-propanol (3:2 v/v) and dried under nitrogen. The dry lipid mixtures were stored at −20 °C and dissolved immediately before sonication in 10 mM NaH_2PO_4 buffer, pH 7.4, containing 1 mM dithiothreitol and 1 mM EDTA. The suspension (total lipid concentration, 0.4 mM) was sonicated for 10 min in an ice bath with a Branson 250 sonicator and then centrifuged for 15 min at 15000 rcf to remove titanium probe particles, multilamellar vesicles, and undispersed lipid (negligible amounts). The final lipid concentration of the donor vesicles per assay was 13 μM, and the final AV-GalCer concentration per assay was 0.13 μM. The POPC acceptor vesicles (total lipid concentration, 10 mM) were prepared by sonication as described above for the sonicated donor vesicles. The lipid concentration of the acceptor vesicles per assay was 130 μM. The final molar ratios of the lipids in the vesicles were routinely checked; phospholipids were analyzed by the method described by Bartlett [23] and cholesterol was analyzed with the cholesterol oxidase-Amplex Red method (Invitrogen). Less than 2% of the lipids were usually changed during sonication and centrifugation vs. the amounts prior to sonication. Light-scattering measurements (Malvern Zetasizer Nano-S ZEN1600, Malvern Instruments, Worcestershire, UK) showed an average vesicle diameter of 45 nm (data not shown).

2.3. Resonance energy transfer assay

The resonance energy transfer assay method for measuring the transfer of glycolipid between donor and acceptor vesicles and the calculations of the transfer rates have been described recently [16], as based on the method described previously [26]. Briefly, the increase in the fluorescence intensity (relief of quenching) as a function of time for AV-GalCer was measured, Fig. 2. The action of GLTP results in a transfer of AV-GalCer from donor to acceptor vesicles, but not the transfer of the quencher, $DiOC_{16}$. Therefore, the distance between the two fluorophores increases beyond their Förster distances, which results in an increase in AV-GalCer fluorescence intensity. The measurements were carried out at 37 °C in the same buffer as was used in the preparation of the vesicles. The excitation and emission spectra of the acceptor, $DiOC_{16}$, and the donor, AV-GalCer, were as reported previously [16].

3. Results

3.1. GLTP-mediated transfer of AV-GalCer from sphingomyelin like matrices

We compared the initial transfer rate of AV-GalCer from different donor vesicle matrices made of SM or SM-like

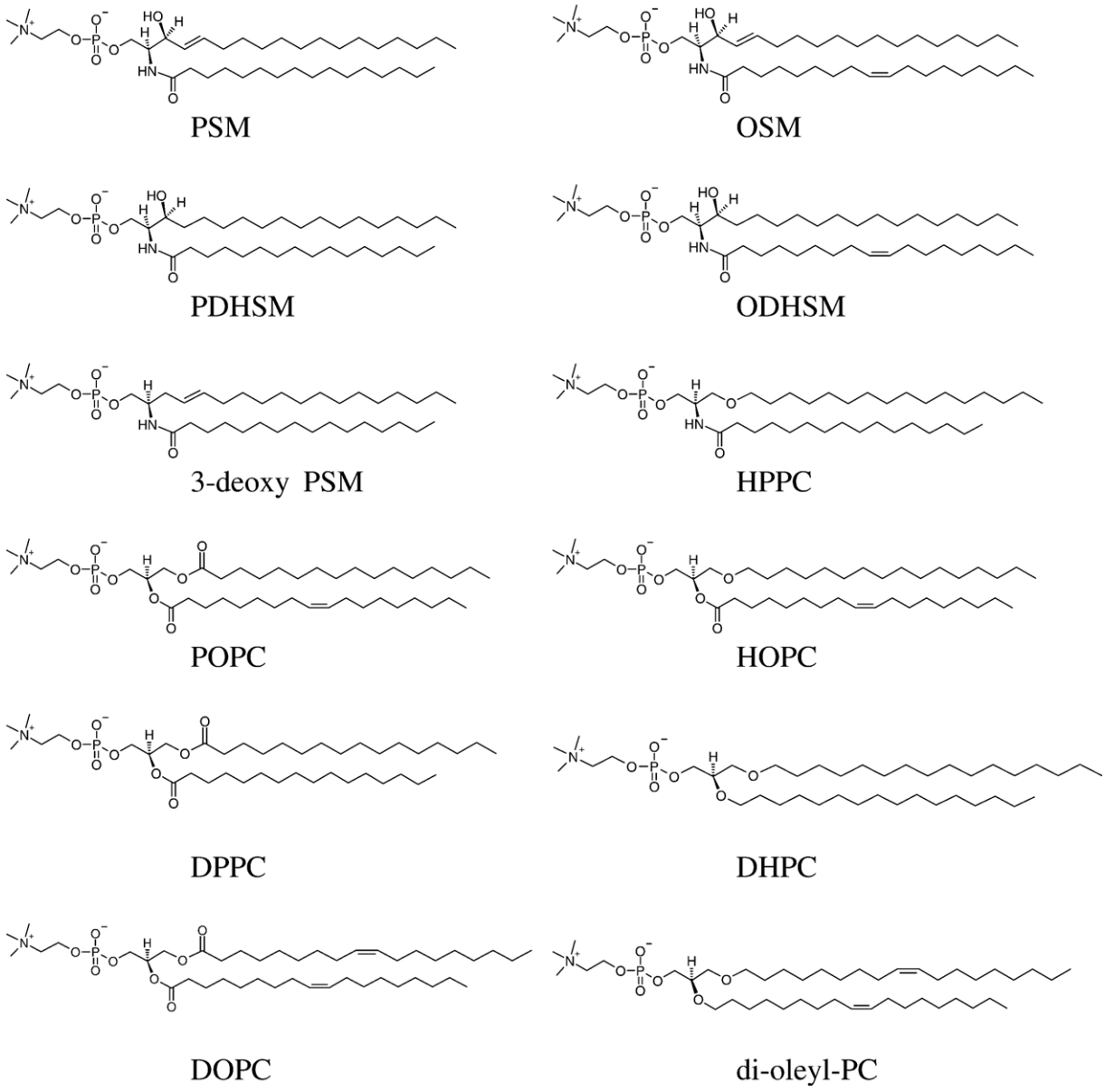

Fig. 1. The structures of the phospholipids used in this study.

analogs. It has been shown that AV-GalCer is not transferred from PSM vesicles and not from PSM/cholesterol mixtures (5–25 mol%) [16]. Fig. 3 shows that AV-GalCer is effectively transferred from OSM vesicles. We also found that the glycolipid can be transferred from vesicles containing 3-deoxy-PSM and, surprisingly, also from PDHSM vesicles, which lack the 3-hydroxy group or the 4,5-*trans* double bond, respectively. The largest rate enhancement was found when a *cis* double bond is present in the amide-linked chain. The transfer rates were 1.0 pmol/s from OSM vesicles, 0.65 pmol/s from deoxy PSM, and 0.3 pmol/s from PDHSM vesicles (Fig. 3). AV-GalCer showed a higher transfer rate from vesicles prepared from the dihydro species, ODHSM. We also used a lipid that is neither sphingosine nor glycerol based, but rather has an ether-linked (hexadecyloxy) chain and an amide-linked (palmitoylamido) chain. AV-GalCer transfer from vesicles prepared from this phospholipid, denoted as HPPC, showed an average rate of 1.2 pmol/s. The AV-GalCer transfer rates from matrices with amide-linked chains are summarized in Fig. 3.

3.2. GLTP-mediated transfer of AV-GalCer from glycerol-based lipid matrices

Fig. 4 illustrates the GLTP-mediated transfer of AV-GalCer from matrices of glycerol-based lipids, having ester or ether bonds at their *sn*-1 and/or *sn*-2 positions. The rate of AV-GalCer transfer is strongly dependent on the unsaturation degree of the acyl and/or alkyl chains. For ester-linked PCs, the transfer rate increased in the order DPPC < POPC < DOPC, having 0, 1, and 2 *cis* double bonds, respectively.

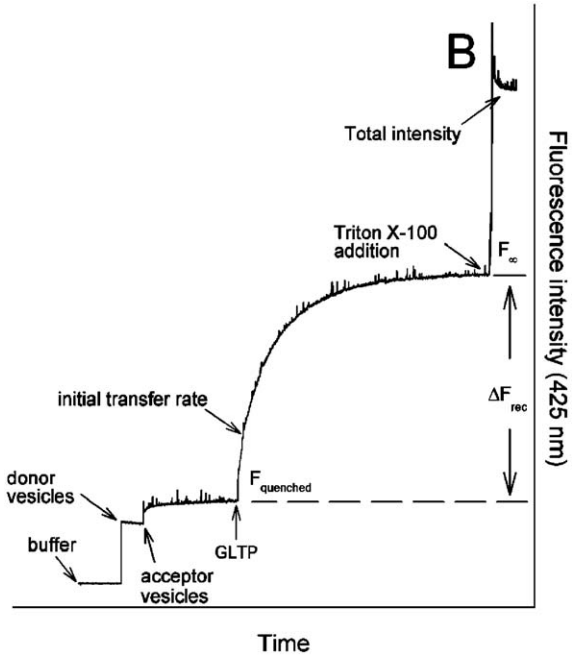

Fig. 2. An illustrative figure of the changes in fluorescence intensity as a function of time during the different steps in the resonance energy transfer assay. The arrows indicate the time points for the respective additions of donor vesicles, POPC acceptor vesicles, GLTP, and Triton X-100.

3.3. GLTP-mediated transfer of AV-GalCer from different ether phospholipid matrices

We used saturated DHPC, and di-oleyl-PC which has one *cis* double bond in each of its 18:1 alkyl chains. We also used a mono-ether PC, HOPC, which has an ester-linked 18:1 chain at the *sn*-2 position and an ether-linked 16:0 chain at the *sn*-1 position. The effect of the degree of unsaturation was similar as observed for ester-PCs, and the total extent of transfer was only slightly affected by the type of bond, ether or ester, at the *sn*-1 and *sn*-2 positions.

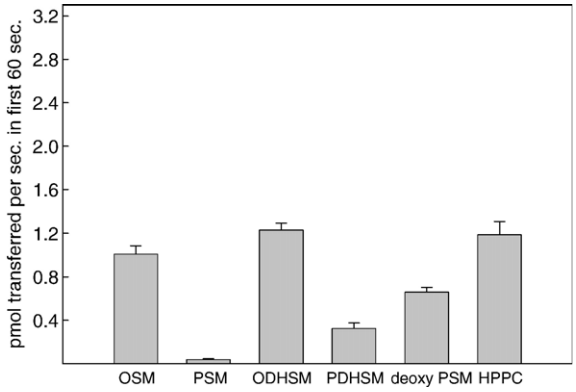

Fig. 3. GLTP-mediated (2 μg) initial AV-GalCer transfer rates from different phospholipid unilamellar vesicles with amide-linked chain-containing lipids. Values are averages and standard deviations of at least four measurements. Significantly different ($P<0.05$), except ODHSM and HPPC.

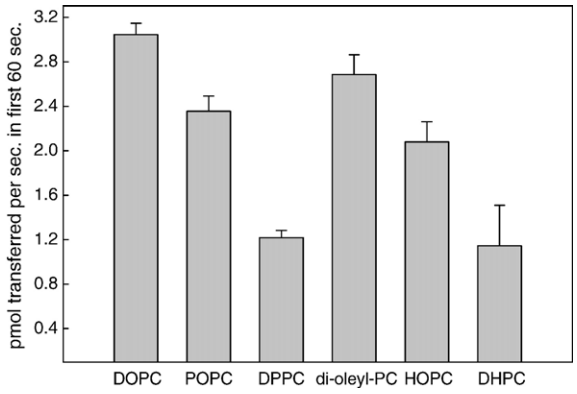

Fig. 4. GLTP-mediated (2 μg) initial AV-GalCer transfer rates from the different phospholipid unilamellar vesicles composed of lipids with ether- and/or ester-linked lipids. Values are averages and standard deviations of at least four measurements. Significantly different ($P<0.05$), except DPPC and dialkyl DHPC, POPC and HOPC.

3.4. Effects of cholesterol AVGalCer transfer

Recently, we showed that the effect of cholesterol on the GLTP-mediated transfer of AV-GalCer from PSM or DPPC matrices was marginal, and only a slight decrease in the transfer rate was observed from POPC vesicles [16]. By measuring the transfer rates from bilayers prepared from OSM with different amounts of cholesterol, we found that the transfer decreased with increasing cholesterol concentrations from 0 to 25 mol%, as seen in Fig. 5.

4. Discussion

It has previously been shown that increasing the SM content in POPC donor vesicles decreased the transfer rate of AV-GalCer in a nonlinear fashion [27] and that AVGalCer is not transferred from PSM vesicles [16]. This is most likely caused by a better lateral mixing and a stronger lipid–lipid interaction between the GSLs and the saturated PSM. Incorporation of cholesterol into POPC vesicles slightly decreased the transfer

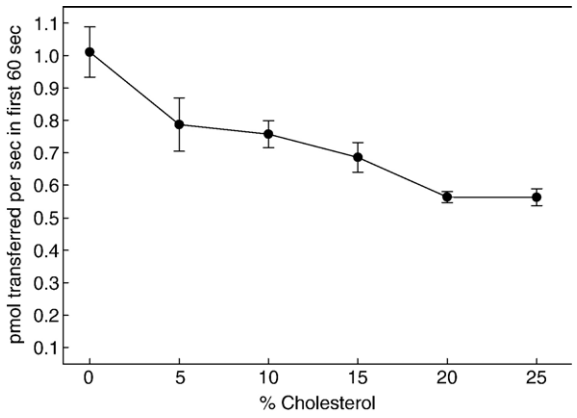

Fig. 5. GLTP-mediated initial AV-GalCer transfer rate from OSM unilamellar vesicles with increasing concentration of cholesterol. Values are averages and standard deviations of at least five measurements. Significantly different ($P<0.05$), except 5% and 10% cholesterol, 20% and 25% cholesterol.

rate, suggesting that the tightly packed liquid-ordered cholesterol/POPC phase allows for a more favorable AV-GalCer mixing, consequently hindering the protein-mediated off rate from the vesicles.

Here, we show that reduction of the 4,5-*trans* double bond increased the transfer rate. With PDHSM, the transfer rate increased from an almost undetectable rate in PSM vesicles to about 0.3 pmol/s; the transfer rate from ODHSM vesicles was slightly increased compared with OSM vesicles. Dihydro-SM has been proposed to form more stable intermolecular hydrogen bonds [28,29], whereas SM is postulated to form more stable intramolecular hydrogen bonds [30]. However, the interaction of DHSM with GSLs is not as well defined. It has been shown that *N*-palmitoyl-GalCer is fully miscible in PSM [18] and bovine-brain SM [31]. Because of the strong intermolecular hydrogen bonds formed in PDHSM the miscibility of AV-GalCer could be somewhat reduced, compared with a better miscibility of AV-GalCer in PSM. If the domains of AV-GalCer formed would afford a greater access for GLTP, an increased transfer rate would be expected. We also found that a SM with a *cis* double bond in its amide chain as the matrix lipid dramatically increased the transfer rate of AV-GalCer. It has previously been shown that OSM promotes less ordered packing in mixtures with cholesterol, which is a consequence of the kink formed by the double bond [32,33]. The less ordered membrane is not a favorable environment for AV-GalCer. The hydroxy group of SM can function as both hydrogen donor and acceptor [34]. Replacing the hydroxy group with a hydrogen atom diminishes this effect, and as a result, the interactions with glycosphingolipids can be expected to decrease.

The transfer rate from the HPPC is approximately the same as that from DHPC; therefore, the amide bond does not affect the kinetics of transfer. In vesicles prepared from PCs, the transfer rate is clearly affected by the extent of saturation in the phospholipid, as is clear for PCs with ester- and ether-bonded acyl/alkyl chains. The 9,10-*cis* double bond in the oleoyl chain results in less ordered lipid packing, and consequently AV-GalCer mixes to a lesser extent with unsaturated PCs, causing a faster off-rate. Cholesterol reduces -gauche isomerizations, generating more liquid-ordered domains in the OSM matrix. The rate of AV-GalCer transfer is decreased with increasing cholesterol amounts, presumably because of a better mixing of AV-GalCer in the liquid-ordered phase. Similar results have been shown previously with POPC vesicles [16].

This study shows that the ability of PSM to reduce the protein-mediated transfer of AV-GalCer has a high degree of structural specificity. GLTP-mediated transfer is hindered only when the glycosphingolipid is surrounded in an environment by a SM with the 4,5-*trans* double bond, the 3-hydroxy group, and a saturated amide chain. The type of bond at the *sn*-1 and *sn*-2 positions of PC (ester, ether, or amide) did not significantly affect the AV-GalCer transfer rate. However, the AV-GalCer transfer rate was sensitive to the degree of saturation in the PC chains, being faster in bilayers formed with more unsaturated, less ordered membranes.

In conclusion, by using different synthetic phospholipid analogs, we examined whether a specific feature of the molecule is responsible for either hampering or promoting the transfer ability of GLTP. We found that GLTP transfers AV-GalCer from bilayers prepared from analogs with structural deviations from that of PSM. It appears that GLTP can more easily transfer AV-GalCer from non-raft like domains than from domains composed of fully saturated SM or SM-resembling lipids that allow for strong lipid–lipid interactions. Introduction of unsaturated and ether lipids into tightly packed domains containing glycosphingolipids would allow GLTP to start to transfer its substrate. It is tempting to speculate that this could have a effect on biological membranes as a regulatory role in GLTP-mediated glycolipid transfer.

Acknowledgments

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References

[1] M.J. Ruocco, D. Atkinson, D.M. Small, R.P. Skarjune, E. Oldfield, G.G. Shipley, X-ray diffraction and calorimetric study of anhydrous and hydrated N-palmitoylgalactosylsphingosine (cerebroside), Biochemistry 20 (1981) 5957–5966.
[2] T.P.W. McMullen, R.N. McElhaney, Physical studies of cholesterol–phospholipid interactions, Curr. Opin. Colloid Interface Sci. 1 (1996) 83–90.
[3] A.H. Futerman, Y.A. Hannun, The complex life of simple sphingolipids, EMBO Rep. 5 (2004) 777–782.
[4] W.C. Byrdwell, D. Borchman, Liquid chromatography/mass-spectrometric characterization of sphingomyelin and dihydrosphingomyelin of human lens membranes, Ophthalmic Res. 29 (1997) 191–206.
[5] R.J. Metz, N.S. Radin, Glucosylceramide uptake protein from spleen cytosol, J. Biol. Chem. 255 (1980) 4463–4467.
[6] A. Abe, K. Yamada, T. Sasaki, A protein purified from pig brain accelerates the intermembrane translocation of mono- and dihexoxylceramides, but not the translocation of phospholipids, Biochem. Biophys. Res. Commun. 104 (1982) 1386–1393.
[7] K. Yamada, T. Sasaki, Rat liver glycolipid transfer protein. A protein which facilitates the translocation of mono- and dihexosylceramides from donor to acceptor liposomes, J. Biochem. 92 (1982) 457–464.
[8] R.E. Brown, F.A. Stephenson, T. Markello, Y. Barenholz, T.E. Thompson, Properties of a specific glycolipid transfer protein from bovine brain, Chem. Phys. Lipids 38 (1985) 79–93.
[9] C.M. Gammon, K.D. Vaswani, R.W. Ledeen, Isolation of two glycolipid transfer proteins from bovine brain: Reactivity toward gangliosides and neutral glycosphingolipids, Biochemistry 26 (1987) 6239–6243.
[10] K. Yamada, A. Abe, T. Sasaki, Glycolipid transfer protein from pig brain transfers glycolipids with beta-linked sugars but not with alpha-linked sugars at the sugar-lipid linkage, Biochim. Biophys. Acta 879 (1986) 345–349.
[11] T.T. Airenne, H. Kidron, Y. Nymalm, M. Nylund, G. West, P. Mattjus, T.A. Salminen, Structural evidence for adaptive ligand binding of glycolipid transfer protein, J. Mol. Biol. 355 (2006) 224–236.

[12] L. Malinina, M.L. Malakhova, A. Teplov, R.E. Brown, D.J. Patel, Structural basis for glycosphingolipid transfer specificity, Nature 430 (2004) 1048–1053.
[13] S.J. Saupe, C. Descamps, B. Turcq, J. Begueret, Inactivation of the *Podospora anserina* vegetative incompatibility locus het-c, whose product resembles a glycolipid transfer protein, drastically impairs ascospore production, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 5927–5931.
[14] P. Brodersen, M. Petersen, H.M. Pike, B. Olszak, S. Skov, N. Odum, L.B. Jorgensen, R.E. Brown, J. Mundy, Knockout of *Arabidopsis* accelerated-cell-death11 encoding a sphingosine transfer protein causes activation of programmed cell death and defense, Genes Dev. 16 (2002) 490–502.
[15] C.S. Rao, T. Chung, H.M. Pike, R.E. Brown, Glycolipid transfer protein interaction with bilayer vesicles: modulation by changing lipid composition, Biophys. J. 89 (2005) 4017–4028.
[16] M. Nylund, P. Mattjus, Protein mediated glycolipid transfer is inhibited FROM sphingomyelin membranes but enhanced TO sphingomyelin containing raft like membranes, Biochim. Biophys. Acta 1669 (2005) 87–94.
[17] P.B. Schneider, E.P. Kennedy, Sphingomyelinase in normal human spleens and in spleens from subjects with Niemann-Pick disease, J. Lipid Res. 8 (1967) 202–209.
[18] Y.J. Björkqvist, T.K. Nyholm, J.P. Slotte, B. Ramstedt, Domain formation and stability in complex lipid bilayers as reported by cholestatrienol, Biophys. J. 88 (2005) 4054–4063.
[19] R. Cohen, Y. Barenholz, S. Gatt, A. Dagan, Preparation and characterization of well defined D-erythro-sphingomyelins, Chem. Phys. Lipids 35 (1984) 371–384.
[20] H.S. Byun, J.A. Sadlofsky, R. Bittman, Enantioselective synthesis of 3-deoxy-(*R*)-sphingomyelin from (*S*)-1-(4′-methoxyphenyl)glycerol, J. Org. Chem. 63 (1998) 2560–2563.
[21] B. Malewicz, J.T. Valiyaveettil, K. Jacob, H.S. Byun, P. Mattjus, W.J. Baumann, R. Bittman, R.E. Brown, The 3-hydroxy group and 4,5-*trans* double bond of sphingomyelin are essential for modulation of galactosylceramide transmembrane asymmetry, Biophys. J. 88 (2005) 2670–2680.
[22] J.G. Molotkovsky, I.I. Mikhalyov, A.B. Imbs, L.D. Bergelson, Synthesis and characterization of new fluorescent glycolipid probes. Molecular organisation of glycolipids in mixed-composition lipid bilayers, Chem. Phys. Lipids 58 (1991) 199–212.
[23] G.R. Bartlett, Phosphorus assay in column chromatography, J. Biol. Chem. 234 (1959) 466–468.
[24] G. West, Y. Nymalm, T.T. Airenne, H. Kidron, P. Mattjus, T.T. Salminen, Crystallization and X-ray analysis of bovine glycolipid transfer protein, Acta Crystallogr., D Biol. Crystallogr. 60 (2004) 703–705.
[25] L. Saunders, J. Perrin, D. Gammack, Ultrasonic irradiation of some phospholipid sols, J. Pharm. Pharmacol. 14 (1962) 567–572.
[26] P. Mattjus, J.G. Molotkovsky, J.M. Smaby, R.E. Brown, A fluorescence resonance energy transfer approach for monitoring protein-mediated glycolipid transfer between vesicle membranes, Anal. Biochem. 268 (1999) 297–304.
[27] P. Mattjus, A. Kline, H.M. Pike, J.G. Molotkovsky, R.E. Brown, Probing for preferential interactions among sphingolipids in bilayer vesicles using the glycolipid transfer protein, Biochemistry 41 (2002) 266–273.
[28] S.R. Ferguson-Yankey, D. Borchman, K.G. Taylor, D.B. DuPre, M.C. Yappert, Conformational studies of sphingolipids by NMR spectroscopy: I. Dihydrosphingomyelin, Biochim. Biophys. Acta 1467 (2000) 307–325.
[29] 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.
[30] C.M. Talbott, I. Vorobyov, D. Borchman, K.G. Taylor, D.B. DuPre, M.C. Yappert, Conformational studies of sphingolipids by NMR spectroscopy: II. Sphingomyelin, Biochim. Biophys. Acta 1467 (2000) 326–337.
[31] D.S. Johnston, D. Chapman, A calorimetric study of the thermotropic behaviour of mixtures of brain cerebrosides with other brain lipids, Biochim. Biophys. Acta 939 (1988) 603–614.
[32] 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.
[33] R.M. Epand, R.F. Epand, Non-raft forming sphingomyelin–cholesterol mixtures, Chem. Phys. Lipids 132 (2004) 37–46.
[34] J.M. Boggs, Intermolecular hydrogen bonding between lipids: influence on organization and function of lipids in membranes, Can. J. Biochem. 58 (1980) 755–770.