Interaction of Cholesterol with Sphingomyelins and Acyl-Chain-Matched Phosphatidylcholines: A Comparative Study of the Effect of the Chain Length

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ABSTRACT In this study we have synthesized sphingomyelins (SM) and phosphatidylcholines (PC) with amide-linked or *sn*-2 linked acyl chains with lengths from 14 to 24 carbons. The purpose was to examine how the chain length and degree of unsaturation affected the interaction of cholesterol with these phospholipids in model membrane systems. Monolayers of saturated SMs and PCs with acyl chain lengths above 14 carbons were condensed and displayed a high collapse pressure (~70 mN/m). Monolayers of *N*-14:0-SM and 1(16:0)-2(14:0)-PC had a much lower collapse pressure (58–60 mN/m) and monounsaturated SMs collapsed at ~50 mN/m. The relative interaction of cholesterol with these phospholipids was determined at 22°C by measuring the rate of cholesterol desorption from mixed monolayers (50 mol % cholesterol; 20 mN/m) to β-cyclodextrin in the subphase (1.7 mM). The rate of cholesterol desorption was lower from saturated SM monolayers than from chain-matched PC monolayers. In SM monolayers, the rate of cholesterol desorption was very slow for all *N*-linked chains, whereas for PC monolayers we could observe higher desorption rates from monolayers of longer PCs. These results show that cholesterol interacts favorably with SMs (low rate of desorption), whereas its interaction (or miscibility) with long chain PCs is weaker. Introduction of a single *cis*-unsaturation in the *N*-linked acyl chain of SMs led to faster rates of cholesterol desorption as compared with saturated SMs. The exception was monolayers of *N*-22:1-SM and *N*-24:1-SM from which cholesterol desorbed almost as slowly as from the corresponding saturated SM monolayers. The results of this study suggest that cholesterol is most likely capable of interacting with all physiologically relevant (including long-chain) SMs present in the plasma membrane of cells.

INTRODUCTION

Sphingomyelins (SM) and phosphatidylcholines (PC) comprise most of the phospholipids in the external plasma membrane leaflet of cells. Both phospholipid classes contain phosphoryl choline as the polar head group, but the backbone of these two phospholipids differ. Sphingomyelin has sphingosine as the hydrophobic backbone, together with an amide-linked acyl chain (Barenholz, 1984). Phosphatidylcholines, on the other hand, are glycerophospholipids with two acyl chains linked to the glycerol backbone with carbonyl ester linkages (Silvius, 1993). Whereas the phosphatidylcholines usually have moderately long acyl chains (palmitic acid in the *sn*-1 position and a mono- or polyunsaturated acyl chain in the *sn*-2 position); the sphingomyelins usually constitute a mixed population with the *N*-linked acyl chain differing widely in length (from 16:0 to $24{:}1^{\Delta 15}$). In addition, PCs are on average much more fluid than SMs, as naturally occurring PCs have on average 1.1 to 1.5 *cis* double bonds per molecule, whereas the corresponding number for SMs is only 0.1 to 0.35 (Barenholz and Thompson, 1980; Barenholz, 1984). Clearly, based on structural differences, the membrane properties of these two choline-containing phospholipids must be very different.

Cholesterol is the major neutral lipid component of the plasma membrane structure in many mammalian cells. In the membrane bilayer, cholesterol influences the physical state and packing density of phospholipids and also modulates the activity of many membrane-associated enzymes, both directly and through effects on phospholipid behavior (Chapman et al., 1969; Demel et al., 1972; Yeagle, 1983, 1985; McMullen and McElhaney, 1996). Whereas cholesterol can influence the behavior of phospholipid acyl chains by interacting with them in the membrane structure; these interactions will also affect the membrane properties of cholesterol itself. This suggestion is supported by the finding that cholesterol desorption from the membrane bilayer is markedly affected by the degree of phospholipid unsaturation, with higher desorption rates observed from unsaturated PCs than from saturated PCs (Phillips et al., 1987; Bittman, 1988). The strength of intermolecular interactions between cholesterol and different phospholipid classes is also known to vary. The rate of cholesterol desorption from a sphingomyelin-containing bilayer is markedly lower as compared with a corresponding phosphatidylcholine system (Fugler et al., 1985; Bhuvaneswaran and Mitropoulos, 1986; Yeagle and Young, 1986; Lund-Katz et al., 1988; Thomas and Poznansky, 1988; Kan et al., 1991). In addition, the compressibility modulus of a cholesterol/sphingomyelin bilayer is much higher than that found in a cholesterol/phosphatidylcholine bilayer (Needham and Nunn, 1990). Both of these effects are likely to result from stronger interaction between cholesterol and sphingomyelin, which may in part be caused by better opportunities of cholesterol to form

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short-distance van der Waals interactions with the long saturated acyl chains commonly found in sphingomyelins (McIntosh et al., 1992). The effect of the phospholipid acyl chain length and unsaturation on cholesterol/phospholipid interactions in monolayers was recently reported for both SMs and PCs (Smaby et al., 1994).

Available evidence suggests that cholesterol appears to interact preferentially with SM in the plasma membranes of cultured cells. A selective degradation of SM is known to result in a partial redistribution of cholesterol within the cells, leading to increased esterification of cholesterol in the endoplasmic reticulum (Slotte and Bierman, 1988; Slotte et al., 1989; Chatterjee, 1993), to a decreased rate of cholesterol biosynthesis (Slotte and Bierman, 1988; Gupta and Rudney, 1991), and to an increased conversion of cholesterol into steroid hormones in steroidogenic cells (Pörn et al., 1991). No such effects are observed when PC is selectively degraded, using PC-specific phospholipase C (Pörn et al., 1993). In addition, a selective degradation of SM results in an increased efflux of cholesterol from cells to β-cyclodextrin, whereas a comparable degradation of plasma membrane PC fails to increase cholesterol efflux (Ohvo et al., 1997). Previously, we also showed that a selective degradation of SM led to an increased cholesterol oxidase susceptibility of plasma membrane cholesterol in cells, whereas a comparable PC degradation did not (Pörn et al., 1993). These results together strongly suggest that cholesterol interacts preferentially with SM in the cell membranes.

Because cell membranes contain SMs with, e.g., 16:0, 18:0, 24:0, as well as 24:1 fatty acids in the *N*-linked position, albeit not necessarily within the same lateral domain, it is of interest to determine how the chain length affects the interactions of cholesterol with the SMs. The objective of this study was to synthesize SMs having *N*-linked acyl chains from 14 to 24 carbons in length (both saturated and monounsaturated fatty acids were used) and to determine how cholesterol interacted with these in monolayer membranes at the air/water interface. Rates of cholesterol desorption from SM monolayers to β-cyclodextrin in the subphase was measured and compared with results obtained with acyl-chain-matched PC monolayers. Our results show that cholesterol appears to interact favorably with saturated SMs, whereas the interaction with long-chain PCs is much weaker.

EXPERIMENTAL PROCEDURES

Materials

Unless otherwise stated, all lipids, β-cyclodextrin (#4767), and cyclohexylamine were obtained from Sigma (St. Louis, MO). Dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) were obtained from Fluka (Buchs, Switzerland). Racemic sphingosylphosphorylcholine (SPC) was obtained from Matreya, Inc. (Pleasant Gap, PA). Fatty acids were purchased from Larodan AB (Malmö, Sweden). 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 cyclodextrin stock solutions were prepared in pure water to a concentration of 40 mM. The water used as subphase 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 MΩcm. The solvents used for synthesis of phospholipids were stored over Molecular Sieves 4A (Merck, Darmstadt, Germany).

Synthesis of *N*-acyl-sphingomyelins

Sphingomyelins with different amide-linked acyl chains were synthesized from racemic sphingosylphosphorylcholine and free fatty-acids essentially as reported by Cohen et al. (1984). In brief, 4.5 μmol of sphingosylphosphorylcholine, 27 μmol of fatty acid, 87 μmol of DCC, and 5 μmol of cyclohexylamine were dissolved in 0.3 ml of dichloromethane containing 9 vol % methanol and a few grains of Molecular Sieves 4A. The reactions were carried out at 40 ± 1°C for 3 h and gave a yield of ≥90%, as determined by thin layer chromatography (TLC). When SMs containing unsaturated fatty acids were prepared, 1 nmol of butylated hydroxytoluene (BHT) was included, and the reactions were carried out under argon to prevent oxidation of the double-bond. The formation of SM was followed by TLC on silica gel G plates, developed with chloroform/methanol/acetic acid/water (25:15:4:2 by vol) according to Skipski et al. (1964). The SMs formed were initially purified using bonded phase columns according to Kaluzny et al. (1985), followed by reversed phase high performance liquid chromatography (HPLC) on a Nucleosil C18 column (5-micron particle size, 150 × 4.6-mm column dimensions, CRS, Inc., Addison, IL) using a flow of 1 ml/min (100% methanol). After these purification steps, the SMs gave a single spot on TLC. The molecular weights of the SMs were determined using matrix-assisted laser desorption mass spectrometry, and the obtained results matched the expected molecular weights for each synthetic product. In addition, the identity of a few of the synthetic products was also positively verified using fast atom bombardment mass spectrometry (FAB-MS). Analysis of the synthetic SMs using high performance TLC HPTLC (elution with chloroform/methanol/acetic acid/water (25:15:4:2 by vol)) revealed the typical double-band pattern of racemic SMs (D-erythro and L-threo; Kronqvist, Leppimäki and Slotte, unpublished data).

Synthesis of phosphatidylcholines

1-Palmitoyl-2-acyl-PCs were synthesized from lyso-PC and free fatty acids by a slight modification of the method of Szolderits et al. (1991). In brief, 4 μmol of 1(16:0)-lyso-PC, 40 μmol of fatty acid, 50 μmol of DCC, and 50 μmol of DMAP were dissolved in 0.5 ml of dry chloroform containing a few grains of Molecular Sieves 4A. The reaction temperature used was ~22°C for synthesis using fatty acids

shorter than 20 carbon atoms. For longer acyl chains 36 ± 2°C was used. Under these conditions the yield was ≥80% after 6 h as determined by TLC developed with chloroform/methanol/water (25:10:1.1). The PCs were purified similarly as described for SMs. After purification, the PCs gave a single spot on TLC analysis.

Force-area isotherms

Pure monolayers of each lipid and mixed monolayers with cholesterol were compressed on water at ambient temperature with a KSV surface barostat (KSV Instruments Ltd., Helsinki, Finland). The barrier speed did not exceed 10 Å^2/molecule per minute during compression. Data were collected using proprietary KSV software. From these isotherms, the mean molecular area value was obtained for a given surface pressure. Information about collapse pressures and phase transitions was also acquired.

Removal of monolayer cholesterol to the subphase by cyclodextrins

Monolayers containing equal molar amounts of cholesterol and phospholipid were prepared at the air/water interface. The trough used was a zero-order type with a reaction chamber (23.9 ml volume, 28.3 cm^2 area) separated by a glass bridge from the lipid reservoir (Verger and de Haas, 1973; Ohvo and Slotte, 1996). The β-CD (in a volume not exceeding 1 ml) was injected into the reaction chamber, the content of which was continuously stirred. The final β-CD-concentration in the subphase was 1.7 mM and the temperature 22°C. The removal of lipids from the monolayer to the subphase was determined from the area decrease of the monolayer at constant surface pressure (20 mN/m) as described by Ohvo and Slotte (1996). The rate of cholesterol desorption was calculated from the total desorption during the first 10 min after injection of β-CD.

RESULTS

Interfacial properties of semisynthetic phospholipids

In this study we have synthesized three different series of phospholipids, a saturated series of sphingomyelins with amide-linked acyl chains ranging from 14 to 24 carbons, a similar unsaturated series of sphingomyelin (14:1 to 24:1 fatty acids), and finally a saturated series of phosphatidylcholines with palmitic acid in the *sn*-1 position and saturated acyl chains (14:0 to 24:0) in the *sn*-2 position. Pure monolayers of these lipids were prepared at the air/water interface using the surface barostat technique. Fig. 1 shows isotherms of pure saturated SM monolayers at 22°C on water. It can be seen that *N*-14:0-SM is slightly less stable than the longer chain SM monolayers, because this monolayer has a lower collapse pressure (~61 mN/m) than the others (~70 mN/m). All of the saturated SMs are fairly condensed, and those with *N*-linked acyl chains of 16 or longer display a phase transition at a fairly low surface pressure (Fig. 1). This finding is consistent with scanning calorimetry analysis of similar SMs, which have showed that these SMs have phase transitions at temperatures close to or above 40°C (Sripada et al., 1987). The monounsaturated SM monolayers gave isotherms without phase transitions at 22°C (Fig. 2) with collapse pressures around 50 mN/m (no clear chain length dependence). A recent mono-

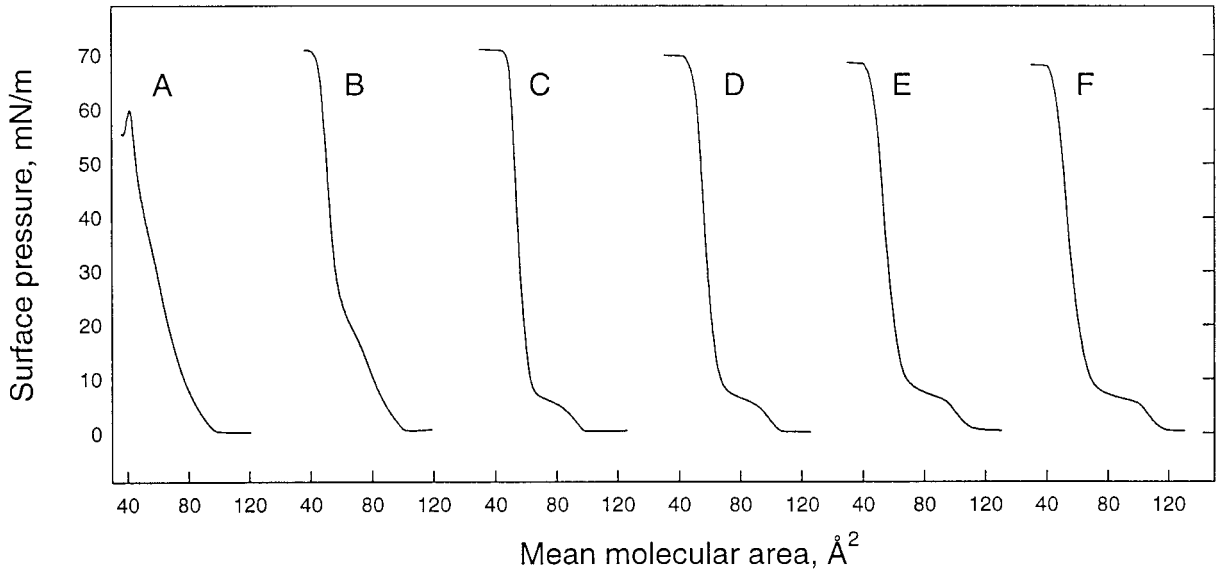

FIGURE 1 Surface pressure versus mean molecular area isotherms for saturated sphingomyelins. *A* represents *N*-14:0-SM, *B* is *N*-16:0-SM, *C* is *N*-18:0-SM, *D* is *N*-20:0-SM, *E* is *N*-22:0-SM, and *F* is *N*-24:0-SM. The pure monolayers were compressed on water at ambient temperature with a speed not exceeding 10 Å^2/molecule per minute.

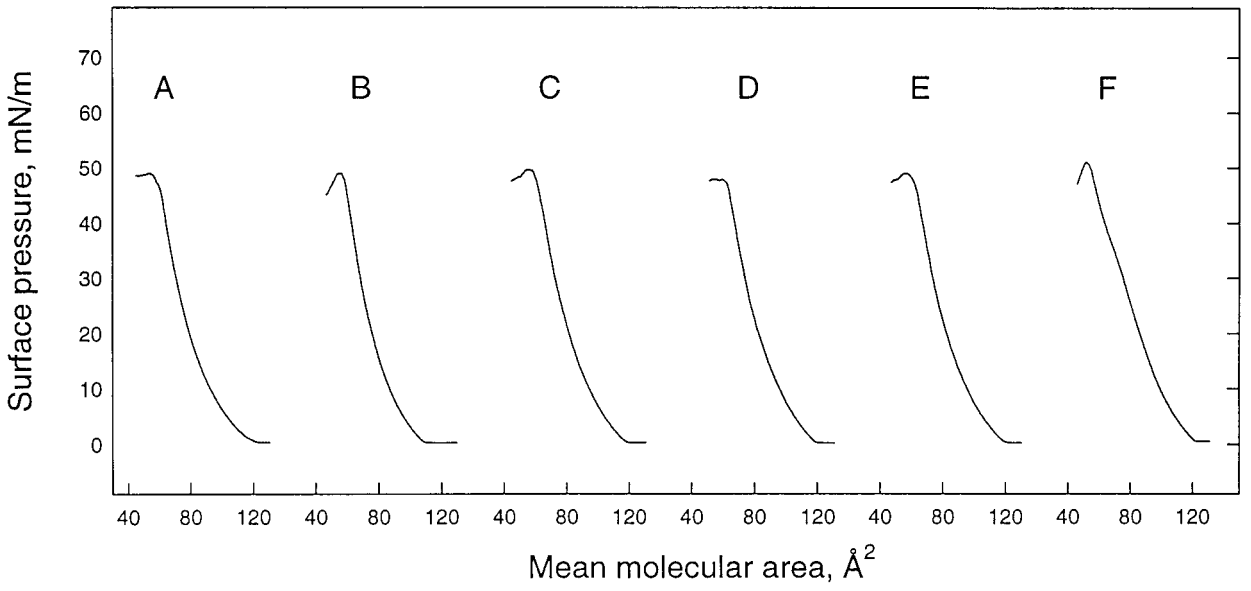

FIGURE 2 Surface pressure versus mean molecular area isotherms for unsaturated sphingomyelins. *A* is *N*-14:1$^{\Delta 9}$-SM, *B* is *N*-16:1$^{\Delta 9}$-SM, *C* is *N*-18:1$^{\Delta 9}$-SM, *D* is *N*-20:1$^{\Delta 11}$-SM, *E* is *N*-22:1$^{\Delta 13}$-SM, and *F* is *N*-24:1$^{\Delta 15}$-SM.

layer study of SMs and PCs describe the interfacial properties of some of the same lipids used in this study (Smaby et al., 1996). The isotherms of saturated 1(16:0)-2(X)-PCs are shown in Fig. 3. As was the case with the corresponding SMs (Fig. 1), the 14:0-analogue had the lowest collapse pressure (~58 mN/m), whereas all the other longer chain PCs had stable monolayers up to ~70 mN/m. 1(16:0)-2(14:0)-PC displayed a phase transition above 30 mN/m, and 1(16:0)-2(16:0)-PC showed a corresponding transition at 5–6 mN/m (Fig. 3). All the other longer chain PCs displayed fully condensed isotherms. Table 1 lists the monolayer phase states of the pure phospholipid monolayers at a lateral surface pressure of 20 mN/m and 22°C (i.e., at the experimental conditions of cholesterol desorption measurements).

Retention on reverse-phase C18 HPLC columns

The semisynthetic SMs and PCs were purified using HPLC and a C18 reverse-phase column. It is well known that the

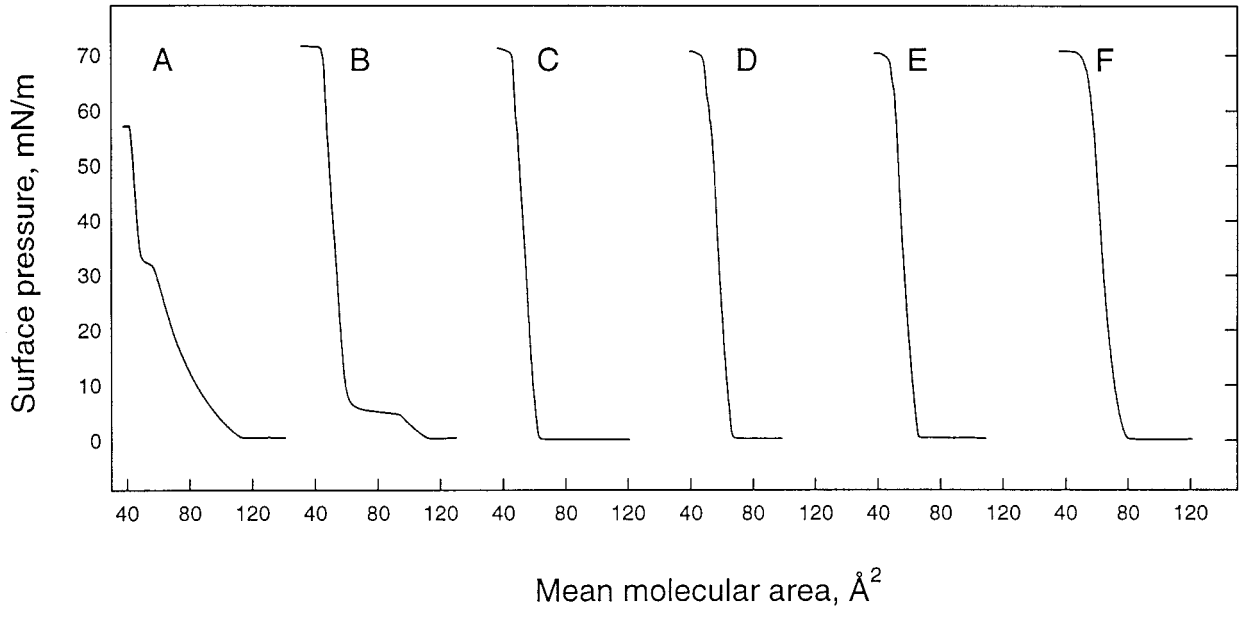

FIGURE 3 Surface pressure versus molecular area isotherms for saturated phosphatidylcholines. *A* represents 1(16:0)-2(14:0)-PC, *B* is 1(16:0)-2(16:0)-PC, *C* is 1(16:0)-2(18:0)-PC, *D* is 1(16:0)-2(20:0)-PC, *E* is 1(16:0)-2(22:0)-PC, and *F* is 1(16:0)-2(24:0)-PC.

TABLE 1 Monolayer phase states of pure phospholipids compressed on water to 20 mN/m at 22°C

Lipid	Phase*	Lipid	Phase	Lipid	Phase
N-14:0-SM	Liquid	*N*-14:1-SM	Liquid	1(16:0)-2(14:0)-PC	Liquid
N-16:0-SM	Mixed	*N*-16:1-SM	Liquid	1(16:0)-2(16:0)-PC	Condensed
N-18:0-SM	Condensed	*N*-18:1-SM	Liquid	1(16:0)-2(18:0)-PC	Condensed
N-20:0-SM	Condensed	*N*-20:1-SM	Liquid	1(16:0)-2(20:0)-PC	Condensed
N-22:0-SM	Condensed	*N*-22:1-SM	Liquid	1(16:0)-2(22:0)-PC	Condensed
N-24:0-SM	Condensed	*N*-24:1-SM	Liquid	1(16:0)-2(24:0)-PC	Condensed

*Phases were derived from analysis of surface pressure versus area isotherms and from surface pressure versus elastic moduli of area compressibility plots (as described by Smaby et al., 1996).

chain length and degree of unsaturation affects retention times on C18 HPLC columns (Sripada et al., 1987), such that longer chain phospholipids have longer retention times than shorter chain analogues. We confirm previous findings and show that our semisynthetic SMs and PCs displayed a retention behavior (given as k'), which was a linear logarithmic function of the chain length (Fig. 4). Our retention analysis also shows clearly that the saturated SMs were more hydrophobic than the chain-length matched PCs, whereas the monounsaturated SMs were less hydrophobic than the saturated SMs or PCs.

Cholesterol desorption from mixed monolayers to β-cyclodextrin in the subphase

The rate of cholesterol desorption is known to be markedly affected by the strength of cholesterol/phospholipid interaction in the donor membrane (for a recent review, see Bittman, 1992). Consequently, cholesterol desorption rate can be used as a measure of how well cholesterol interacts with, e.g., SMs or PCs in a mixed monolayer (Ohvo and Slotte, 1996). The benefit of using β-cyclodextrins is that the measurable desorption rate is increased markedly, and desorption studies that otherwise would take hours to complete can now be completed in ~10 min (Bittman, 1992; Ohvo and Slotte, 1996). The lateral distribution of sphingomyelin and cholesterol in biological membranes apparently is very heterogeneous (Brown and Rose, 1992; Schroeder et al., 1996). Because of this heterogeneity, and because of the apparent co-localization of SM and cholesterol (Pörn et al., 1993; Ohvo et al., 1997), we have examined cholesterol efflux from monolayers containing a fairly high proportion of cholesterol, i.e., 50 mol % cholesterol.

As shown in Fig. 5, the rate of cholesterol desorption

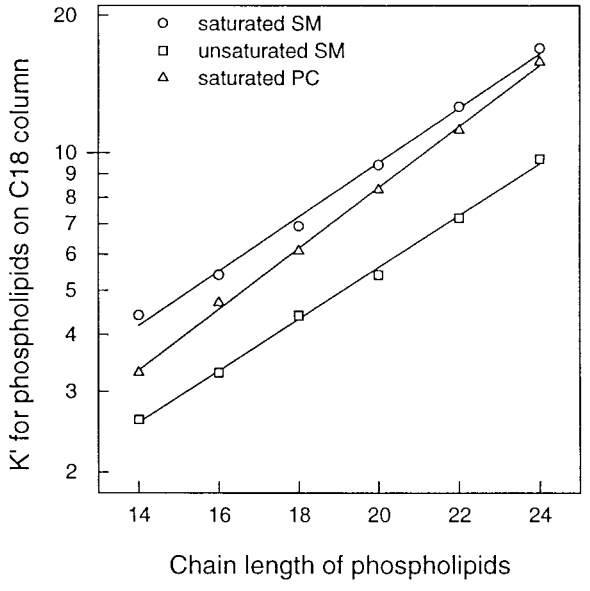

FIGURE 4 Analysis of phospholipid retention on a reverse-phase high-pressure liquid chromatography column. The semisynthetic phospholipids were injected into a Nucleosil C18 HPLC column and were eluted with methanol (1 ml/min flow) at room temperature. The k' values indicated were calculated according to $k' = (t - t_o)/t_o$ in which t is the retention time of the sample and t_o is that of the solvent.

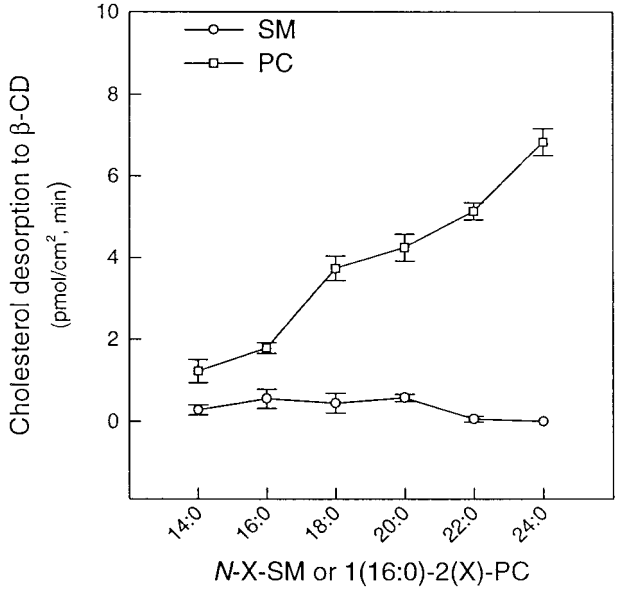

FIGURE 5 Desorption of cholesterol from mixed cholesterol/phospholipid monolayers to β-cyclodextrin in the subphase. Mixed monolayers containing 50 mol % cholesterol and either PCs or SMs were prepared at the air/water interface. The monolayers were compressed to 20 mN/m and maintained at a constant surface pressure (at ambient temperature). Cholesterol desorption to β-CD (1.7 mM) in the subphase was determined as a time function and calculated values are plotted as a function of the acyl chain length of *N*-acyl-SM (−○−) or 1(16:0)-2(X)-PC (−□−). The values are given as the mean ± SE from three different monolayer experiments.

from mixed monolayers to β-cyclodextrin in the subphase (at 1.7 mM) was markedly lower from saturated SM monolayers than from chain-length-matched PC monolayers. The difference in desorption rate for SM and PC monolayers was moderate for the shorter chain phospholipids but became very large as the chain length increased. With phospholipids containing 24:0 acyl chains, cholesterol desorption was at least 10 times faster from PC than from SM monolayers (Fig. 5). The rate of cholesterol desorption from monounsaturated SM monolayers is shown in Fig. 6. Clearly, the rate of desorption decreases as the chain length of the SM increases. However, the position of the single *cis*-double also changes from Δ^9 for 14:1, 16:1, and 18:1, to Δ^{11} for 20:1, Δ^{13} for 22:1, and Δ^{15} for 24:1. It is also likely that cholesterol interaction with the SM becomes better as the *cis*-bond moves farther away from the interface. When the results for saturated and unsaturated SMs are compared, one can observe that the cholesterol desorption rate was much higher from monounsaturated SMs than from saturated SMs when the chain length was 20 or shorter. Interestingly, cholesterol desorption from 22:1 SM and 24:1 SM was almost as slow as from saturated SMs, suggesting that the double bond at the Δ^{13} or the Δ^{15} position did not weaken the interaction of cholesterol with the phospholipid.

DISCUSSION

This work represents a systematic monolayer study of semisynthetic SMs and PCs with defined acyl chains ranging from 14 to 24 in length. Special emphasis was laid on how the chain length in the phospholipids affected the interaction of cholesterol with them in monolayer membranes. The selection of acyl chains was physiologically relevant for the SMs (Barenholz, 1984) but not for the PCs, which normally do not have long saturated acyl chains in their *sn*-2 position. However, the saturated PC series was synthesized to function as chain-matched controls for the saturated series of SMs. The longer chain (saturated) phospholipids (≥16 carbons per chain) all displayed a condensed (chain ordered) phase at surface pressures relevant to biological membranes (above 20 mN/m). All of the monounsaturated SMs were liquid-expanded at 20 mN/m. When the interaction of cholesterol with saturated SMs and PCs is compared, it is important to note that for long-chain analogues, the phase states for SMs and PCs were similar.

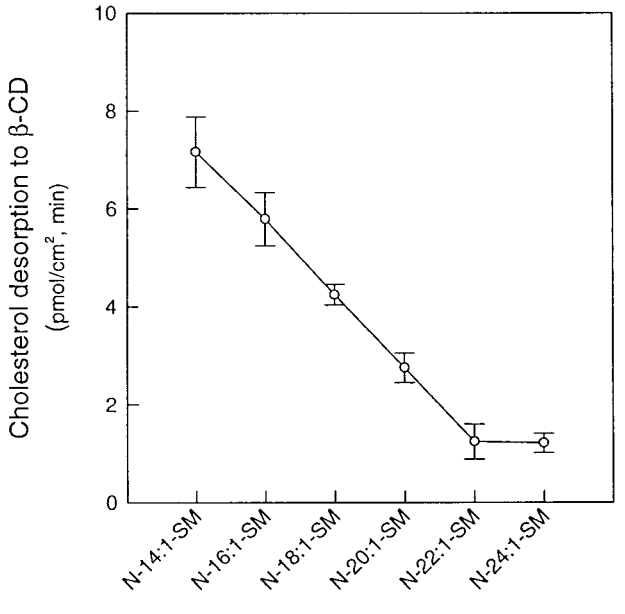

FIGURE 6 Desorption of cholesterol from mixed cholesterol/sphingomyelin monolayers to β-cyclodextrin in the subphase. Mixed monolayers containing 50 mol % cholesterol and unsaturated SMs were prepared at the air/water interface. The monolayers were compressed to 20 mN/m and maintained at a constant surface pressure (at ambient temperature). Cholesterol desorption to β-CD (1.7 mM) in the subphase was determined as a time function and calculated values are plotted as a function of the unsaturated acyl chain length of *N*-acyl-SM. The values are given as the mean ± SE from three different monolayer experiments.

As a measure of cholesterol/phospholipid interaction, the rate of cholesterol desorption from monolayers was examined. This rate is markedly affected by the extent of van der Waals attractive interactions between cholesterol and the acyl chains of the phospholipids. Spontaneous cholesterol desorption from model membranes is a fairly slow process with measured half-times being in the range of hours rather than minutes (Phillips et al., 1987; Bittman, 1992). The relatively slow desorption rate is related to the fairly high activation energy needed for cholesterol desorption to acceptor membranes (20 kcal/mol), which results from the fact that cholesterol has to pass trough the water phase on its way from a donor membrane to an acceptor particle (Phillips et al., 1987). However, the rate of cholesterol desorption can be increased substantially by using β-cyclodextrins as acceptors, because these apparently can pick up cholesterol from the membrane interface and thus decrease the necessary activation energy for desorption to only 7–9 kcal/mol (Yancey et al., 1996). We have previously successfully used β-cyclodextrin to effect both cholesterol (Ohvo and Slotte, 1996) and fatty acid (Slotte and Illman, 1996) desorption from monolayers to the subphase and correlated the desorption rates with intermolecular interactions in the monolayer membranes.

Cholesterol desorption rates measured from monolayers containing different phospholipids clearly show that cholesterol interacts very differently with chain-matched SMs and PCs. Cholesterol desorption from SM monolayers was always slower than from comparable PC monolayers. Interestingly, the rate of cholesterol desorption increased as the PC *sn*-2 chain length increased, whereas the rate was constantly low for all *N*-linked acyl chain lengths in SM. The desorption results from the PC monolayers can be interpreted to suggest that cholesterol phase separated to some extent in the longer chain PC environment (because of hydrophobic mismatch). Phase separation of lipids in model membranes is favored by the mismatch of the hydrophobic interaction surfaces of the interacting lipids (Mabrey and Sturtevant, 1976; van Dijck et al., 1977; Lee, 1977; Silvius et al., 1996). The propensity of cholesterol to possibly phase separate in one phospholipid system (PC) but not in the

other (SM) was not because of differences in the phase states of the monolayers, as both the long chain PCs and the long chain SMs were condensed and chain ordered at the given lateral surface pressure and temperature.

Our previous studies of cholesterol/phospholipid interactions in monolayers have shown that SM has a larger capacity to solubilize cholesterol as compared with the capacity of PC (Slotte, 1992). It is possible that the hydroxyl group of cholesterol can form hydrogen bonds with the amide function in SM, thereby stabilizing the interaction even above equimolar cholesterol concentrations. This interpretation is supported by recent model membrane studies in which we have shown that when the *N*-linked acyl chain in SM is replaced by a carbonyl ester-linked acyl chain, the interaction of cholesterol with the SM decreases dramatically as determined by its cholesterol oxidase susceptibility (Bittman et al., 1994).

The phase state of the monolayers was apparently not a major determinant of cholesterol desorption rates, as both saturated SM and PC monolayers were condensed and chain ordered (at 18 or longer acyl chains) at the experimental conditions of the desorption experiments. In addition, with monounsaturated SMs, the desorption of cholesterol from long-chain SM (22 and 24 carbons) was as slow as from the saturated counterpart SM monolayer, although the pure monounsaturated monolayers were in a liquid state at 20 mN/m and 22°C. However, the position of the single *cis*-double bond in *N*-22:1$^{\Delta 13}$-SM and *N*-24:1$^{\Delta 15}$-SM is apparently so far away from the interface that it does not overlap with the region in the SM molecule that cholesterol interacts with (McIntosh et al., 1992). The situation is obviously quite different with SM species having Δ^9 unsaturated fatty acids in the *N*-linked position. With these SMs, the double bond position directly interferes with cholesterol/acyl chain interactions, resulting in faster desorption rates as compared with SM species lacking the *cis*-double bond or where the position is distal to the interaction region of cholesterol. It is also not likely that differences in the extent of cholesterol-induced phospholipid condensation could result in different desorption rates for the SM and PC monolayers, as Smaby and co-workers recently demonstrated that cholesterol condenses SMs and PCs equally if these are acyl-chain matched (Smaby et al., 1994), as is the case in this study.

To conclude, we have determined that cholesterol interacts favorably with SMs, even when their *N*-linked chains are long (e.g., 24 carbons). Cholesterol does not interact as well with asymmetric PCs where the *sn*-2 acyl chain is equally long. Biological membranes are known to contain SMs with highly saturated both moderate length and long *N*-linked acyl chains (Barenholz, 1984; Brown and Rose, 1992). The results of this study imply that cholesterol potentially can interact with all physiologically relevant SM species present in membranes. This finding is of biological importance as it has been suggested that sphingolipids (including SM) and cholesterol form microdomains or rafts in the membranes of cells (for reviews, see Simons and Ikonen, 1997; Brown and London, 1997). A recent model membrane study, in which the interactions of cholesterol, sphingomyelin, and GM1 gangliosides was measured using high-sensitivity calorimetry, indicated that cholesterol/SM-rich phases segregated from SM/GM1-ganglioside-rich phases. These results show that lipid properties alone can lead to segregation of specific lipid domains in the plane of the membrane, and more interestingly, that cholesterol appeared to favor interacting with SM (Ferraretto et al., 1997). We have previously shown that in ternary-mixed monolayers containing cholesterol, phosphatidylcholine, and sphingomyelin, cholesterol interacts preferentially with SM over PC (Mattjus and Slotte, 1996). Our recent experiments with plasma membranes in intact cells also indicate that cholesterol preferentially interacts with SM over PC (Pörn et al., 1993; Ohvo et al., 1997), clearly implying that cholesterol/SM interactions have important consequences on the structure and function of biological membranes in general and on specific SM-rich lateral domains in particular.

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