Interaction of Sphingomyelins and Phosphatidylcholines with Fluorescent Dehydroergosterol†

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ABSTRACT: The fluorescent sterol dehydroergosterol was used as a cholesterol analogue in conjunction with multifrequency phase and modulation (1–250 MHz) fluorometry to examine whether sterols (1) interact preferentially with fluid- or solid-phase phospholipids and (2) interact preferentially with sphingomyelin in phase-separated or phase-miscible cosonicated phospholipid membranes. Cosonicated small unilamellar vesicles (SUV) were produced by mixing lipids in organic solvents, drying the mixture, adding buffer, sonicating, and separating SUV. Phospholipids of synthetic as well as biological origin were utilized. In phase-separated, cosonicated SUV of dimyristoylphosphatidylcholine/distearoylphosphatidylcholine (DMPC/DSPC, 1:1 molar ratio), the fluorescent sterol (0.5 mol %) interacted preferentially with the fluid-phase lipid (partition coefficient, $K_{f/s}$ = 2.6–3.4) according to four criteria. First, dehydroergosterol detected only the phase transition of DMPC, the phospholipid with the lower phase transition temperature. Second, the dehydroergosterol fluorescence polarization, limiting anisotropy, order parameter, and rotational relaxation time in the cosonicated vesicle were similar to those of dehydroergosterol in SUV composed only of DMPC. Third, the number of dehydroergosterol fluorescence lifetime components as well as the distribution in the cosonicated SUV was similar to that of dehydroergosterol in SUV composed of DMPC. Fourth, dehydroergosterol concentration-dependent self-quenching was detected in DSPC SUV at much lower dehydroergosterol concentration than in DMPC SUV. Preference of dehydroergosterol for fluid-phase lipids was also observed by monitoring dehydroergosterol exchange between individually sonicated DMPC SUV and DSPC SUV after the two types of vesicles were mixed in equal proportions. In these SUV mixtures, the dehydroergosterol also partitioned into the more fluid SUV, 99:1. In contrast, in largely phase-miscible, cosonicated DMPC/DPPC SUV, the fluorescence properties of dehydroergosterol did not indicate a preferential interaction with either phospholipid. In a variety of phase-separated, cosonicated sphingomyelin/phosphatidylcholine SUV of either synthetic (palmitoyloleoylphosphatidylcholine/stearoylsphingomyelin and palmitoyloleoylphosphatidylcholine/palmitoylsphingomyelin) or biological (egg phosphatidylcholine/bovine brain sphingomyelin) origin, the fluorescent sterol also interacted preferentially with the lipid with lower phase transition temperature, palmitoyloleoylphosphatidylcholine ($K_{f/s}$ greater than 13) or egg phosphatidylcholine ($K_{f/s}$ = 2.4–3.2). When the sphingomyelin was the component with the lower phase transition temperature (e.g., DSPC/palmitoylsphingomyelin SUV), the dehydroergosterol interacted preferentially with the sphingomyelin ($K_{f/s}$ = 2.9–3.1). In phase-miscible cosonicated phosphatidylcholine/sphingomyelin SUV (DMPC/palmitoylsphingomyelin), no preference for specific phospholipids was evident. In the phase-separated cosonicated phosphatidylcholine/sphingomyelin SUV, there was some evidence for fluid–fluid-phase immiscibility.

Recent interest in sphingomyelin has focused on the potential preferential interaction of this lipid with cholesterol [reviewed in Barenholz (1984), Schroeder (1984b), and Schroeder and Nemecz (1989)]. Such an interaction may in part account for the nonuniform intra- as well as intermembrane distribution of cholesterol (Schroeder, 1984a; Schroeder & Nemecz, 1989). Preferential interaction of sphingomyelin with cholesterol also appears important to reverse cholesterol transport (Stein et al., 1988) and to microsomal cholesterol esterification (Slotte & Bierman, 1988). However, the interaction of sphingomyelin with cholesterol is still unclear. Theoretical calculations (Vandenheuval, 1963) as well as some calorimetry data in model membranes (Demel et al., 1977) suggest that cholesterol interacts preferentially with sphingomyelin in lipid mixtures exhibiting lateral phase separation and in which sphingomyelin had either the higher or the lower phase transition temperature. However, cholesterol–sphingomyelin complex formation did not occur in phase-miscible mixtures (Untracht & Shipley, 1977; Calhoun & Shipley, 1977). Using exchange experiments, other investigators also showed that there was no preference between sphingomyelin and dipalmitoylphosphatidylcholine, two lipids with identical acyl chains (Lange et al., 1979).

The above observations may in part be reconciled by the presence of other factors affecting cholesterol distribution, e.g., differences in acyl chain composition between the phosphatidylcholine and the sphingomyelin or differences in phase state of the two lipids (Yeagle & Young, 1986). Indeed, some differential scanning calorimetry evidence indicates that, with the exception of sphingomyelin (Demel et al., 1977), cholesterol preferentially associated with the phospholipid component of lower phase transition temperature in a variety of phase-separated phospholipid bilayers (van Dijck et al., 1977; De Kruijff et al., 1973, 1974; Demel et al., 1977). Cholesterol

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exchange experiments between small unilamellar vesicles (SUV)[1] or LUV[1] also indicated that a membrane in the gel state is a poor cholesterol acceptor (Yeagle & Young, 1986). Since sphingomyelin usually has a higher phase transition temperature than phosphatidylcholine, one might expect preferential interaction of sterol with the latter lipid in phase-separated systems. However, even above the phase transition temperature of both bovine brain sphingomyelin vesicles and palmitoyloleoylphosphatidylcholine (POPC)[1] vesicles, little transfer of cholesterol to POPC vesicles took place (Yeagle & Young, 1986).

These data indicate the necessity for systematic studies of factors affecting cholesterol distribution not only between but also within membranes. In the present investigation, both natural and synthetic sphingomyelins, phosphatidylcholines, and a naturally occurring fluorescent cholesterol analogue (dehydroergosterol) are used to investigate potential sterol–phospholipid interactions in phase-separated as well as phase-miscible phospholipid mixtures in SUV.

Experimental Procedures

Reagents. 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC),[1] dimyristoylphosphatidylcholine (DMPC),[1] and distearoylphosphatidylcholine (DSPC)[1] were purchased from Avanti Biochemical Inc. (Birmingham, AL). *N*-Palmitoylsphingomyelin (PSP),[1] *N*-stearoylsphingomyelin (SSP),[1] and bovine brain sphingomyelin (brain SP)[1] were from Sigma Chemical Co. (St. Louis, MO). These lipids were checked for purity by thin-layer chromatography and stored in sealed ampules under N_2 at −70 °C. Dehydroergosterol, although a natural product (Sica et al., 1982; Delseth et al., 1979), was synthesized and purified by high-performance liquid chromatography as described previously (Fischer et al., 1984, 1985a,b). Purity was confirmed by high-performance liquid chromatography, absorbance peak ratios, and comparison with dehydroergosterol standards obtained from FRANN Scientific Inc. (Columbia, MO).

Preparation of Liposomes. Small unilamellar vesicles (SUV)[1] were prepared as previously described (Schroeder et al., 1987, 1988a,b; Nemecz & Schroeder, 1988; Nemecz et al., 1988). POPC, DSPC, DPPC, DMPC, egg phosphatidylcholine, SSP, PSP, bovine brain sphingomyelin, and their mixtures were dissolved in chloroform or ethanol in the proportions indicated in the table or figure legends. Unless stated otherwise, dehydroergosterol (0.5 mol %) was added, the samples were placed in solvent-cleaned glass tubes and evaporated under N_2, and trace amounts of solvents were removed in vacuo for 24 h. A buffer containing 10 mM PIPES and 0.02% NaN_3, pH 7.4, in deionized distilled water was filtered through a 0.2-μm membrane (Millipore Inc., Bedford, MA) and added at 24 °C or above the phase transition temperature of the lipid to provide a final lipid concentration of 4 mM. The samples were vortexed and sonicated for 3 min with an American Beauty S/70 150-W Ultrasonic bath (Branson Cleaning Equipment Co., Shelton, CT), to remove the lipid from the sides of the tube and yield a milky suspension of multilamellar vesicles. This suspension was then further sonicated with five 3-min bursts using a titanium microtip probe and a W-300 Sonic Dismembrator (Fisher Scientific Inc., Pittsburgh, PA) set at 35% maximum output. Sonication was always performed under N_2 and above the phase transition temperature of the matrix phospholipid. The resulting SUV were separated from large vesicles and multilamellar liposomes by differential ultracentrifugation for 2 h with a 40 Ti rotor and an L7-55 ultracentrifuge (Beckman Instruments, Fullerton, CA).

Lipid Composition. The lipid composition of the SUV was determined as follows: Total phospholipid concentration was estimated by determination of phospholipid phosphorus (Ames, 1968). The relative mole percent of phosphatidylcholine and sphingomyelin was determined after lipid extraction, two-dimensional thin-layer chromatography, visualization with I_2 vapor, elution, and phosphate assay (Schroeder et al., 1987). The mole percent dehydroergosterol was determined by using ergosterol as an internal standard prior to lipid extraction, and separation of sterols from phospholipids by silicic acid chromatography. Sterols were resolved by high-performance liquid chromatography as described earlier (Fisher et al., 1984, 1985a). Alternately, the neutral lipid fraction from silicic acid column chromatography was dissolved in ethanol, and the fluorescence of the neutral lipids in ethanol was determined at 324-nm excitation (376-nm emission) and compared to the fluorescence intensity of a standard curve of dehydroergosterol in ethanol.

Fluorescence Spectroscopy. Steady-state fluorescence polarization, *P*, was determined by using a T-format SLM 4800 (SLM Instruments, Inc., Urbana, IL) updated to 1–250-MHz multifrequency phase and modulation capability (ISS Instruments, Champaign, IL). In determination of *P*, light scattering was reduced by using narrow band-passes in the excitation monochromator and cutoff filters in the emission system. In addition, samples were serially diluted, and *P* was measured extrapolated to zero absorbance (Lentz et al., 1979). Steady-state anisotropy, *r*, was measured as described earlier (Schroeder et al., 1987).

Fluorescence lifetimes were measured in the L format by 1–250-MHz multifrequency phase and modulation fluorometry with the above instrument. This instrument is based on the cross-correlation principle introduced by Spencer and Weber (1969) and is described in detail elsewhere (Gratton & Limkeman, 1983). A He/Cd laser (Model 4240NB, Liconix, Sunnyvale, CA), whose emission intensity at 325 nm was modulated sinusoidally with a Pockels cell, was the light source. Fluorescence emission was observed through a GC-375 sharp cutoff filter (Janos Technology Inc., Townshend, VT) to eliminate scattered light. Fluorescence lifetimes were measured at 14 different modulation frequencies with the excitation polarizer set at 0° and the emission polarizer set at the magic angle, 55°, in order to eliminate Brownian motion as a determinant of apparent lifetime and to eliminate polarization effects in general. Fluorescence lifetime was measured relative to a reference solution of dimethyl-POPOP (Chemalog Chemical Dynamics Corp., South Plainfield, NJ) in absolute ethanol at 24 °C (lifetime = 1.45 ns) as described previously (Lakowicz et al., 1981). Since fluorescence lifetime is very sensitive to changes in light scattering, especially due to aggregation or fusion of vesicles (Schullery et al., 1980), great care was exercised to ensure that scattered light was not present or that it was eliminated as described above for steady-state polarization measurements. The turbidity of the SUV described herein was constant over the time of the experiments, and light scattering was insignificant even for SUV preparations that were over 14 days old. Data were collected by a Compaq PC computer with an ISS-ADC interface (ISS Instruments Inc., Champaign, IL).

[1] Abbreviations: egg PC, egg phosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; brain SP, brain sphingomyelin; SSP, *N*-stearoylsphingomyelin; PSP, *N*-palmitoylsphingomyelin; SUV, small unilamellar vesicle(s); LUV, large unilamellar vesicle(s); dehydroergosterol, $\Delta^{5,7,9(11),22}$-ergostatetraen-3β-ol.

Arrhenius plots of dehydroergosterol fluorescence polarization were obtained in the T format as described earlier (Sweet et al., 1987). The polarization values were corrected for the grating correction factor. Data were obtained in both ascending and descending temperature scans every 1 °C over the range 5–65 °C. Only descending temperature data are presented herein.

Lifetime distributional analysis was performed as described earlier (Fiorini et al., 1987; Schroeder et al., 1988a,b; Nemecz & Schroeder, 1988). The data were fitted to uniform, Gaussian, or Lorentzian distributions. The latter provided the best fits. Each lifetime Lorentzian distribution was characterized by a center of distribution, C, a fractional intensity, F, and a distributional width, W, at peak half-height. For bimodal distributions, two such sets of parameters were obtained. The reduced χ^2 parameter was utilized as described by Fiorini et al. (1987) to judge the quality of fit. The statistical analyses used for the fitting procedure do not attribute physical significance to the parameters but only that the data fit the model used.

Differential polarized phase fluorometry was used over a range of 14 different frequencies between 1 and 250 MHz to obtain the rotational relaxation time (nanoseconds) and limiting anisotropy of dehydroergosterol in phospholipid SUV according to the procedure developed by Weber (1978) and Lakowicz et al. (1979). Details of the procedure are given elsewhere (Schroeder et al., 1987; Sweet et al., 1987; Nemecz & Schroeder, 1988). In the curve-fitting procedure, a fixed value of r_0 (the anisotropy in the absence of rotational motion) of 0.385 was used (Schroeder et al., 1987). The rotational relaxation time (nanoseconds) equals 6 × rotational rate [(in radians/s)$^{-1}$]. The lipid order parameter, S, was measured according to the expression S = (limiting anisotropy/r_0)$^{1/2}$ as described elsewhere (Heyn, 1979).

Determination of Dehydroergosterol Partition Coefficient between Phospholipid Phases. The partition coefficient of dehydroergosterol between phospholipid phases was calculated according to the equations developed by Lentz et al. (1976) except that dehydroergosterol was used instead of 1,6-diphenyl-1,3,5-hexatriene. Lifetime values for these calculations were obtained as described above. For lipid mixtures in which no preferential interaction was observed, the partition coefficients were near 1.

Results

Phase Behavior of Pure and Cosonicated Phospholipid Vesicles. In order to determine if sterols interact preferentially with fluid- or solid-phase lipids, dehydroergosterol was chosen as a fluorescent sterol analogue to probe the partitioning of sterols between coexisting fluid (liquid-crystalline) and solid (gel) phases in phospholipid SUV. A series of pure as well as phase-miscible and phase-separated phospholipids have been identified by a variety of techniques (Van Dijck et al., 1977; de Kruijff et al., 1974; Barenholz et al., 1976; Calhoun & Shipley, 1979; Untracht & Shipley, 1979). In the present as well as earlier investigations, it was assumed that all the vesicles have the average composition, i.e., demixing does not occur. Preferential interaction of cholesterol in such systems was examined as described in the following example: POPC, the principal component of egg yolk phosphatidylcholine, has a phase transition far below physiological temperature, −5 °C (Untracht & Shipley, 1979). In contrast, DSPC is in the solid or gel phase up to 52.8 °C. In cosonicated phospholipid mixtures that are phase-separated, temperatures may be chosen at which both lipids are fluid, one lipid is in the fluid phase while the other lipid is in the gel phase, or both lipids

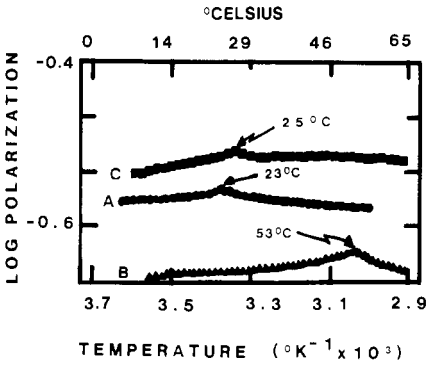

FIGURE 1: Temperature dependence of dehydroergosterol fluorescence polarization in phosphatidylcholine SUV. Dehydroergosterol (0.5 mol %) was incorporated into (C) dimyristoylphosphatidylcholine [DMPC (●)], (B) distearoylphosphatidylcholine [DSPC (▲)], and (A) cosonicated DMPC/DSPC [1:1 molar ratio (■)] small unilamellar vesicles (SUV) as described under Experimental Procedures. Steady-state fluorescence polarization was determined as a function of descending temperature also as described therein.

are in the gel phase. A small amount (0.5 mol %) of the fluorescent sterol, dehydroergosterol, is incorporated into these SUV. Due to concentration-dependent self-quenching and sterol lateral-phase separation above 6 mol % sterol, such experiments are not possible at higher mole percent sterol (Ipsen et al., 1987; Schroeder et al., 1987, 1988a,b; Rogers et al., 1979). The dynamic (rotational relaxation time and lifetime) and static (limiting anisotropy and order parameter) characteristics as well as steady-state polarization (Arrhenius plots) of 0.5 mol % dehydroergosterol differ in each SUV comprised of only a single phospholipid species. Thus, when the above parameters are determined at each temperature in the pure phospholipid SUV as well as cosonicated phospholipid SUV, preferential interactions of the dehydroergosterol with the gel or liquid-crystalline phase as well as preferential interaction with sphingomyelin or phosphatidylcholine may be resolved.

Distribution of Dehydroergosterol between Fluid and Solid Phospholipid Phases in Cosonicated Phosphatidylcholine Vesicles. Dehydroergosterol (0.5 mol %) in phospholipid liposomes composed of DMPC (curve C) or DSPC (curve B) detects a single phase transition near 25 ± 2 and 53 ± 3 °C (n = 3), respectively, as ascertained by Arrhenius plots of steady-state polarization (Figure 1). These results confirm those reported with Arrhenius plots of dehydroergosterol polarization (Chong & Thompson, 1986; Smutzer & Yeagle, 1985), lifetime (Smutzer et al., 1986), and absorbance-corrected fluorescence intensity (Kier et al., 1986) in DMPC SUV and of absorbance-corrected fluorescence intensity in DSPC SUV (Kier et al., 1986). The transition temperatures observed with dehydroergosterol are also close to those reported by other techniques not using fluorescent sterol probes, namely, 23 and 54 °C (Janiak et al., 1976; Silvius et al., 1979). The majority of reports regarding the gel-phase miscibility of DMPC and DSPC in cosonicated SUV indicated that regions of gel-phase immiscibility, possibly with peritectic phase behavior, exist in this system [reviewed in Mason (1988)]. However, some overlap of the transitions occurs (Van Dijck et al., 1977; de Kruijff et al., 1974). Therefore, in cosonicated SUV, these lipids are expected to be both in the solid gel phase and in the fluid liquid-crystalline phase at temperatures below 23 °C and above 54 °C, respectively. At intermediate temperatures (37 °C), the lipids are phase-separated: the DMPC is in the fluid phase while the DSPC is in the solid phase. In order for the results described below to be valid, structural alterations in the cosonicated SUV must not occur in the time frame of the

Table I: Effect of Lateral Phase Separation on the Distribution of Dehydroergosterol in Phosphatidylcholine Vesicles[a]

preparation	temp (°C)	polarization	limiting anisotropy	rotational relaxation time (ns)	order parameter
DMPC	10	0.329 ± 0.010	0.268 ± 0.009	0.89 ± 0.05	0.83 ± 0.04
DSPC	10	0.270 ± 0.020	0.134 ± 0.013	1.05 ± 0.01	0.59 ± 0.03
DMPC/DSPC	10	0.384 ± 0.001	0.266 ± 0.005	0.75 ± 0.10	0.83 ± 0.01
DMPC	37	0.326 ± 0.010	0.163 ± 0.018	0.71 ± 0.17	0.65 ± 0.05
DSPC	37	0.254 ± 0.007	0.098 ± 0.020	1.21 ± 0.21	0.50 ± 0.06
DMPC/DSPC	37	0.310 ± 0.011	0.127 ± 0.015	0.81 ± 0.13	0.58 ± 0.04
DPPC	10	0.386 ± 0.009	0.276 ± 0.010	0.54 ± 0.16	0.85 ± 0.04
DMPC/DPPC	10	0.385 ± 0.011	0.250 ± 0.020	1.12 ± 0.21	0.81 ± 0.05
DPPC	37	0.342 ± 0.015	0.204 ± 0.021	0.94 ± 0.11	0.73 ± 0.03
DMPC/DPPC	37	0.315 ± 0.011	0.185 ± 0.009	0.69 ± 0.21	0.69 ± 0.04

[a] Values represent the mean ± SEM (n = 3–4). Molar ratios of DMPC/DSPC and DMPC/DPPC are 1:1.

Table II: Dehydroergosterol Partitioning between Phase-Separated Phospholipids in Cosonicated SUV[a]

phospholipid, X/Y	parameter	partition coefficient (X/Y)
DMPC/DSPC	polarization	3.4 ± 0.6
	limiting anisotropy	2.6 ± 0.4
POPC/PSP	polarization	13+
	limiting anisotropy	13+
POPC/SSP	polarization	13+
	limiting anisotropy	13+
egg PC/brain SP	polarization	2.4 ± 0.3
	limiting anisotropy	3.2 ± 0.5
PSP/DSPC	polarization	2.9 ± 0.5
	limiting anisotropy	3.1 ± 0.4

[a] All partition coefficients were determined at 37 °C as described under Experimental Procedures with the exception of DSPC/PSP which was determined at 45 °C.

experiments. The fluorescence polarization of dehydroergosterol at 37 °C in DMPC, DSPC, and cosonicated SUV was 0.326, 0.254, and 0.310, respectively. These values were constant with time over the period 24–48 h.

An Arrhenius plot of dehydroergosterol fluorescence polarization in cosonicated DMPC/DSPC SUV detects only the phase transition near 23 ± 1 °C (curve C, Figure 1). The dynamic and structural properties of dehydroergosterol indicate preferential partitioning of dehydroergosterol into the fluid-phase lipid, i.e., DMPC (Table I). At 10 °C, a temperature below the phase transition temperatures of DSPC and DMPC, the fluorescence polarization, limiting anisotropy, and order parameter of dehydroergosterol resemble those of the phospholipid with the lower phase transition temperature, DMPC. Likewise, at temperatures above the phase transition of DMPC but below that of DSPC (e.g., 37 °C), the above parameters for dehydroergosterol either more closely resemble those of the DMPC or are intermediate between those of pure DMPC and pure DSPC. Partition coefficients of dehydroergosterol between DMPC and DSPC, calculated from polarization and limiting anisotropy values obtained at 37 °C, were 3.4 and 2.6, respectively (Table II), also indicating preferential interaction of dehydroergosterol with DMPC. At temperatures above both phase transitions (65 °C), the fluorescence polarization, limiting anisotropy, and order parameter of dehydroergosterol more closely resembled those in DMPC, possibly indicating the presence of some fluid–fluid-phase immiscibility of the two phospholipids in the liquid-crystalline phase.

The above results were confirmed by lifetime distributional analysis (Figure 2). At 10 °C, dehydroergosterol had a narrow lifetime distribution in DMPC SUV best described as a single component, C_1, centered near 1.927 ns with a half-height peak width, W_1, of 0.077 ns, χ^2 = 3. In DSPC SUV, the dehydroergosterol had two components: C_1 = 1.372 ns, W_1 = 0.331 ns, F_1 = 0.67; C_2 = 3.726 ns, W_2 = 0.05 ns, χ^2 = 5. In contrast, in the cosonicated DMPC/DSPC SUV at 10 °C, dehydroergosterol displayed primarily a single broad lifetime distribution with a single C_1 = 1.893 ns, W_1 = 0.206 ns, χ^2 = 4 (Figure 2). Thus, at 10 °C, the dehydroergosterol preferentially interacted with DMPC, the phospholipid with the lower phase transition temperature. However, the broadening of the lifetime distribution indicates that some miscibility of the two phospholipid species may occur. The data observed both at 37 °C and at 65 °C (Figure 2, χ^2 ranged between 1 and 4) are also consistent with dehydroergosterol preferentially interacting with DMPC, the phospholipid with the lower phase transition temperature. It should be noted that with increasing temperature, the dehydroergosterol lifetimes shortened and the distribution became narrower: in DMPC, C_1 was 1.927, 0.671, and 0.325 ns while W_1 was 0.077, 0.05, and 0.05 ns at 10, 37, and 65 °C, respectively. Similar shifts were observed in the DSPC center of lifetime distribution, C_1, and peak width at half-height, W_1, and in the cosonicated mixture. At all temperatures, the lifetime and distributional width of dehydroergosterol resembled those in the more fluid DMPC. The observation that at 65 °C, a temperature at which both lipids are expected to be in the liquid-crystalline phase, the dehydroergosterol in the cosonicated SUV does not exhibit the longer lifetime attributable to the DSPC environment is also consistent with some fluid-phase immiscibility of the DMPC and DSPC.

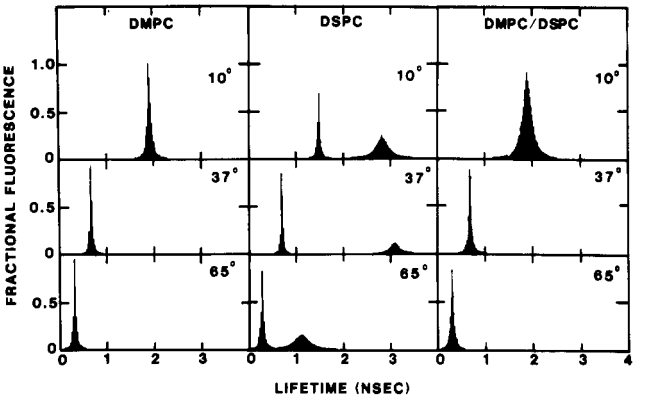

FIGURE 2: Lifetime distributional analysis of dehydroergosterol in phosphatidylcholine SUV. Dehydroergosterol (0.5 mol %) was incorporated into DMPC, DSPC, and DMPC/DSPC (1:1 molar ratio) SUV, and fluorescence lifetime was determined at the indicated temperatures as described under Experimental Procedures. The data were analyzed for two-component Lorentzian distributions also as described under Experimental Procedures.

The above experiments were repeated using SUV composed of cosonicated phosphatidylcholines that do not display a large phase separation, DMPC and dipalmitoylphosphatidylcholine (DPPC)[1] (Lentz et al., 1976; Demel et al., 1977). In fact,

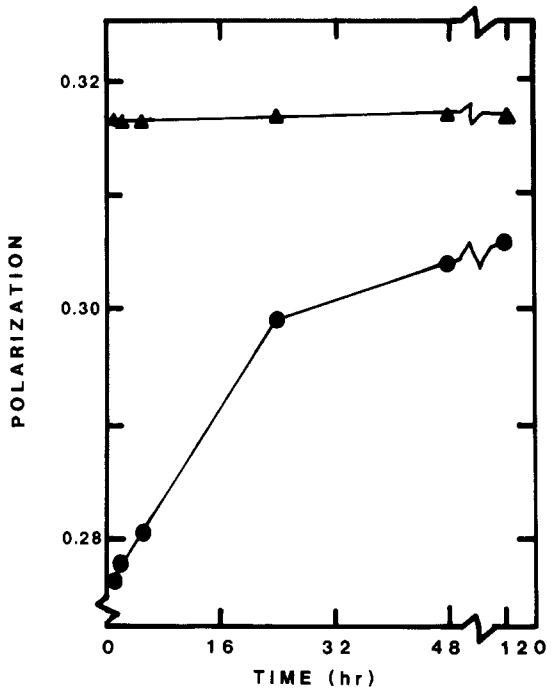

FIGURE 3: Exchange of dehydroergosterol between mixed dimyristoylphosphatidylcholine and distearoylphosphatidylcholine SUV. Dehydroergosterol (0.5 mol %) was incorporated into donor DMPC SUV and mixed with a 10-fold excess of acceptor DSPC SUV [top curve (▲)]. Alternately, the dehydroergosterol was incorporated into donor DSPC SUV and mixed with a 10-fold excess of acceptor DMPC SUV [bottom curve (●)]. Exchange of dehydroergosterol between the mixed vesicles was monitored at 37 °C by measuring the steady-state polarization as a function of time.

these lipids are apparently phase-separated only in a very narrow temperature range. Dehydroergosterol detected the phase transition in DMPC SUV [Figure 1; see also Chong and Thompson (1986), Smutzer and Yeagle (1985), Smutzer et al. (1986), and Kier et al. (1986)] and DPPC SUV (Rogers et al., 1979; Chong & Thompson, 1986). As expected, a phase transition for the cosonicated DMPC/DPPC SUV was not detected by dehydroergosterol (data not shown). In addition, in cosonicated vesicles composed of DPPC and DMPC, the fluorescence properties of dehydroergosterol do not indicate a preferential interaction with either phosphatidylcholine (Table I). At temperatures below the phase transition of either phospholipid (10 °C) and at temperatures intermediate between the phase transitions of both phospholipids (37 °C), the fluorescence parameters of the sterols were intermediate.

In summary, dehydroergosterol displays preferential interaction with the more fluid phospohatidylcholine when the phosphatidylcholines in a cosonicated vesicle are phase-separated. DMPC/DSPC-cosonicated SUV demonstrate gel-phase immiscibility as well as immiscibility at temperatures intermediate between the two phase transition temperatures. The data are also consistent with some liquid-crystalline-phase immiscibility of these two phosphatidylcholines.

Distribution of Dehydroergosterol between Mixed Fluid- and Solid-Phase Phosphatidylcholine SUV. In order to confirm the preferential interaction of dehydroergosterol with fluid-phase phosphatidylcholine, pure phospholipid SUV composed of either DMPC or DSPC were mixed rather than cosonicated. The donor vesicles contained 0.5 mol % dehydroergosterol. The fluorescence polarization of dehydroergosterol in DMPC SUV versus DSPC SUV differed markedly. Polarization was higher in DMPC than in DSPC SUV (Figure 3 and Table I). The basis for this difference was discussed earlier (Fischer et al., 1985a). When the donor

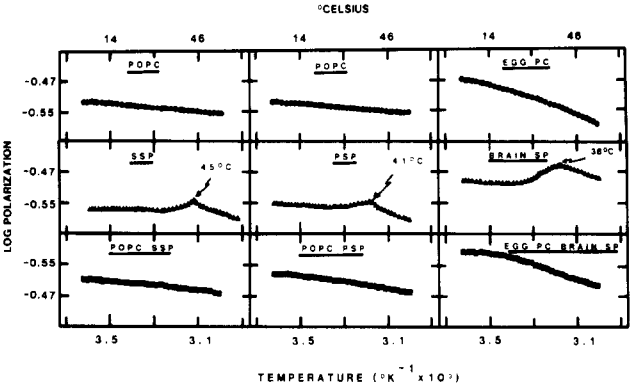

FIGURE 4: Temperature dependence of dehydroergosterol fluorescence polarization in cosonicated phosphatidylcholine/sphingomyelin SUV. All procedures were performed as described in the legend to Figure 1 except that phosphatidylcholine (●), sphingomyelin (▲), and cosonicated phosphatidylcholine/sphingomyelin (■) SUV were utilized. The phospholipids used were palmitoyloleoylphosphatidylcholine (POPC), egg phosphatidylcholine (egg PC), stearoylsphingomyelin (SSP), palmitoylsphingomyelin (PSP), and bovine brain sphingomyelin (brain SP).

and acceptor vesicles were DMPC/dehydroergosterol and DSPC, respectively, and exchange was performed at a temperature intermediate between the phase transitions (37 °C), exchange was not measurable (Figure 3). When the opposite experiment was performed, i.e., the donor and acceptor SUV were DSPC/dehydroergosterol and DMPC, respectively, exchange occurred very slowly at 37 °C (Figure 3). Partition coefficients of dehydroergosterol in a mixed DSPC/DMPC system and a DMPC/DSPC system were 1 and 99, respectively, at 37 °C. In summary, when the donor vesicle was more fluid DMPC, dehydroergosterol did not partition into solid-phase DSPC acceptor vesicles. In contrast, when the donor vesicle was the more rigid DSPC, dehydroergosterol partitioned slowly into the more fluid DMPC. Thus, dehydroergosterol preferentially interacts with fluid-phase phosphatidylcholine acceptor SUV.

Distribution of Dehydroergosterol between Fluid and Solid Phases in Cosonicated Sphingomyelin/Phosphatidylcholine Vesicles. As indicated by Arrhenius plots of fluorescence polarization over the range 5–65 °C, dehydroergosterol detects the phase transition of stearoylsphingomyelin (SSP), palmitoylsphingomyelin (PSP), and brain sphingomyelin (brain SP) near 45 ± 3, 41 ± 2, and 38 ± 1 °C (n = 3), respectively (Figure 4). Phase transitions of palmitoyloleoylphosphatidylcholine (POPC) and egg phosphatidylcholine (egg PC) are not detected in this temperature range (Figure 4). POPC/SSP and egg PC/brain SP are known to form phase-separated mixtures (Barenholz et al., 1976; Untracht & Shipley, 1979). It is clear from the Arrhenius plots of dehydroergosterol in cosonicated POPC/SSP, POPC/PSP, and egg PC/brain SP that a phase transition is not detected (Figure 4). Thus, dehydroergosterol preferentially interacts not with sphingomyelin but instead with the phosphatidylcholine which has the lower phase transition temperature. This observation was also supported by dynamic (rotational relaxation time) and static (limiting anisotropy and order parameter) characteristics, as well as steady-state polarization of dehydroergosterol (Table III). In cosonicated SUV at intermediate temperatures where both phases coexist (37 °C), the dehydroergosterol polarization, limiting anisotropy, order parameter, and rotational relaxation time resembled those of dehydroergosterol in POPC, the phospholipid with the lower phase transition temperature, more than those of dehydroergosterol in SSP. Partition coefficients of dehydroergosterol

Table III: Dynamic and Static Properties of Dehydroergosterol in POPC/SSP and DMPC/PSP Liposomes[a]

preparation	temp (°C)	polarization	limiting anisotropy	rotational relaxation time (ns)	order parameter
POPC	37	0.318 ± 0.011	0.121 ± 0.005	0.98 ± 0.11	0.56 ± 0.03
SSP	37	0.273 ± 0.011	0.106 ± 0.003	1.45 ± 0.14	0.52 ± 0.02
POPC/SSP	37	0.316 ± 0.006	0.163 ± 0.006	0.89 ± 0.09	0.65 ± 0.03
POPC	65	0.278 ± 0.013	0.105 ± 0.006	1.03 ± 0.09	0.52 ± 0.05
SSP	65	0.187 ± 0.016	0.039 ± 0.009	1.28 ± 0.11	0.32 ± 0.04
POPC/SSP	65	0.278 ± 0.009	0.118 ± 0.011	0.89 ± 0.15	0.55 ± 0.05
DMPC	37	0.326 ± 0.011	0.162 ± 0.010	0.71 ± 0.08	0.65 ± 0.04
PSP	37	0.281 ± 0.019	0.142 ± 0.020	1.05 ± 0.15	0.61 ± 0.13
DMPC/PSP	37	0.309 ± 0.012	0.170 ± 0.016	0.87 ± 0.09	0.66 ± 0.05

[a] Molar ratios of POPC/SSP and DMPC/PSP are 1:1. Values represent the mean ± SEM (n = 3–4).

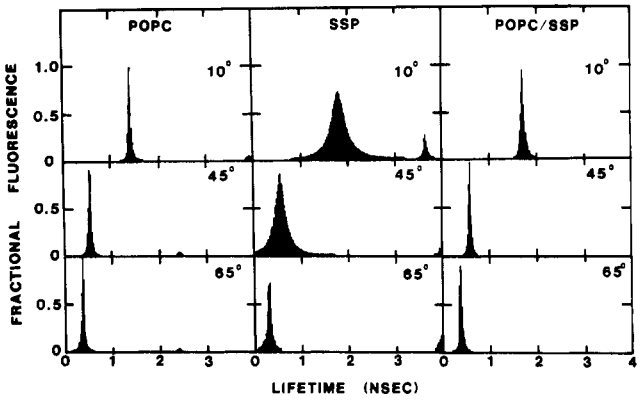

FIGURE 5: Dehydroergosterol fluorescence lifetime distribution in palmitoyloleoylphosphatidylcholine and stearoylsphingomyelin SUV. All procedures were as described for Figure 3 except that palmitoyloleoylphosphatidylcholine (POPC), stearoylsphingomyelin (SSP), and POPC/SSP cosonicated SUV containing 0.5 mol % dehydroergosterol were used.

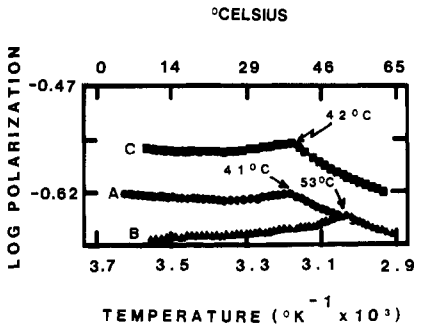

FIGURE 6: Temperature dependence of dehydroergosterol polarization in cosonicated phospholipid SUV in which sphingomyelin has the lower phase transition temperature. All procedures were the same as described in the legend to Figure 1 except (A) palmitoylsphingomyelin (PSP), (B) distearoylphosphatidylcholine (DSPC), and (C) palmitoylsphingomyelin/distearoylsphingomyelin (PSP/DSPC) were used.

between POPC and SSP were in excess of 13, clearly in favor of the more fluid POPC (Table II). Above the phase transition temperatures of both lipids (65 °C), dehydroergosterol still preferentially interacted with the POPC, indicating fluid–fluid-phase immiscibility.

The above results were confirmed by lifetime distributional analysis (Figure 5). At 10 °C, dehydroergosterol had a narrow lifetime distribution best described as two components, the major of which was C_1 = 1.5 ns, W_1 = 0.07 ns, F_1 = 0.98, and χ^2 = 1.8. In SSP, dehydroergosterol displayed two broader components: C_1 = 1.8 ns, W_1 = 0.4 ns, and F = 0.73 and C_2 = 3.6 ns, W_2 = 0.07 ns, and χ^2 = 4. In the cosonicated SUV at 10 °C, primarily a narrow lifetime distribution (C_1 = 1.6 ns, W_1 = 0.05 ns, F_1 = 0.99, and χ^2 = 6) was observed (Figure 5). Thus, at 10 °C, the dehydroergosterol preferentially interacted with POPC, the phospholipid with the lower phase transition temperature. The lack of broadening of the POPC lifetime distribution in the cosonicated SUV indicates nearly complete gel-phase immiscibility of the two phospholipid species. The data obtained both at 45 °C and at 65 °C are also consistent with dehydroergosterol preferentially interacting with POPC, the phospholipid with the lower phase transition temperature. The observation that at 65 °C, a temperature above the phase transitions of both POPC and SSP, the dehydroergosterol does not exhibit either of the broad lifetime distributions of SSP in the cosonicated SUV is also consistent with some fluid-phase immiscibility of the POPC and SPP. A similar fluid–fluid-phase immiscibility was observed with dehydroergosterol in POPC/PSP vesicles at 65 °C (data not shown).

The above experiments were repeated using SUV composed of cosonicated phosphatidylcholine and sphingomyelin that are not phase-separated. Dehydroergosterol detects the phase transitions of DMPC and PSP near 25 ± 2 °C (Figure 1) and 41 ± 2 °C (Figure 4), respectively. These two phospholipids, however, form a phase-miscible mixture in cosonicated vesicles (Calhoun & Shipley, 1979). In Arrhenius plots of dehydroergosterol polarization in cosonicated DMPC/PSP SUV, a phase transition was not detected (data not shown). In addition, at a temperature intermediate between the transition temperatures of both lipids (37 °C), dehydroergosterol displays no significant differences in polarization, limiting anisotropy, or order parameter in the DMPC/PSP-cosonicated SUV as compared to the pure phospholipid SUV (Table III).

The preference of dehydroergosterol for fluid versus solid phases was examined in SUV comprised of phospholipids in which sphingomyelin was the lipid with lower phase transition temperature. Dehydroergosterol detects the phase transition of palmitoylsphingomyelin (PSP, curve A, Figure 6) and of distearoylphosphatidylcholine (DSPC, curve B, Figure 6) near 41 ± 2 and 53 ± 3 °C, respectively. In cosonicated mixtures (curve C, Figure 6) of the two lipids, only the transition near 42 °C was apparent, indicating a preference of dehydroergosterol for the more fluid PSP. These results were confirmed by comparison of static and dynamic properties of dehydroergosterol in these vesicles. In cosonicated SUV, the fluorescence polarization, limiting anisotropy, order parameter, and rotational relaxation time of the fluorescent sterol again resembled those of the dehydroergosterol in the more fluid PSP both above and below the phase transition temperatures of the two lipids (not shown). At intermediate temperatures, the partition coefficient of dehydroergosterol between PSP and DSPC was 2.9 and 3.1 in favor of the more fluid lipid, as determined from polarization and limiting anisotropy data, respectively (Table II).

Last, phase-separated cosonicated SUV composed of naturally occurring phospholipids were used to determine if the results obtained with synthetic lipids could be extended to lipids of biological origin. Dehydroergosterol senses a phase transition in bovine brain sphingomyelin (brain SP) near 38 ± 1

Table IV: Dynamic and Static Properties of Dehydroergosterol in Egg Phosphatidylcholine/Bovine Brain Sphingomyelin Liposomes[a]

preparation	temp (°C)	polarization	limiting anisotropy	rotation relaxation time (ns)	order parameter
egg PC	10	0.330 ± 0.008	0.206 ± 0.009	0.73 ± 0.10	0.73 ± 0.04
bovine brain SP	10	0.402 ± 0.016	0.286 ± 0.010	0.81 ± 0.08	0.86 ± 0.06
egg PC/bovine brain SP	10	0.376 ± 0.010	0.263 ± 0.012	0.76 ± 0.11	0.82 ± 0.03
egg PC	45	0.270 ± 0.010	0.165 ± 0.016	0.54 ± 0.08	0.66 ± 0.04
bovine brain SP	45	0.321 ± 0.009	0.194 ± 0.011	0.72 ± 0.06	0.71 ± 0.03
egg PC/bovine brain SP	45	0.282 ± 0.012	0.165 ± 0.009	0.59 ± 0.11	0.65 ± 0.05

[a] Molar ratio of egg PC/bovine brain SP is 1:1. Values represent the mean ± SEM (n = 3–4).

°C but not in egg phosphatidylcholine (egg PC) over the range 5–65 °C (Figure 4). Egg PC does, however, have a phase transition near −5 °C (Untracht & Shipley, 1979). Egg PC and bovine brain SP form a phase-separated mixture (Untracht & Shipley, 1979). Arrhenius plots of dehydroergosterol polarization in cosonicated egg PC/bovine brain SP do not detect the transition of the sphingomyelin near 38 °C (Figure 4). The results in Table IV indicate that at intermediate temperature (10 °C) between the phase transitions, the static (limiting anisotropy and order parameter), dynamic (rotational rate), and steady-state polarization of dehydroergosterol are intermediate between those of individual phospholipid species. At temperatures above 45 °C (above the phase transitions of both lipids), the static and dynamic parameters (Table IV) as well as lifetime (not shown) resemble those of the more fluid lipid, egg PC, consistent with fluid–fluid-phase immiscibility. Thus, in cosonicated SUV comprised of naturally occurring phospholipids, the fluorescent sterol does not display preferential affinity for sphingomyelin. In fact, above both transition temperatures, dehydroergosterol displays a preference for egg PC, the phospholipid with the lower phase transition temperature. The latter finding is also consistent with fluid–fluid-phase immiscibility in this system.

Discussion

Cholesterol may potentially interact with specific phospholipid(s). Such interactions may be important both to the asymmetric distribution of lipids between membranes and within (lateral as well as transbilayer) membranes as well as to cholesterol transport and associated membrane functions as outlined in the introduction. A major impediment to advances in the area of sterol–phospholipid interactions has been the lack of suitable probe molecules that, when inserted into membranes or lipoproteins, accurately reflect the behavior of cholesterol [reviewed in Schroeder (1984b)]. More recently, fluorescence analogues of cholesterol have been popularized [reviewed in Schroeder (1984b, 1988), Schroeder and Nemecz (1989), and Yeagle (1985)]. Of these molecules, dehydroergosterol has proven most useful as a membrane cholesterol analogue as indicated by results of extensive comparisons of the physical properties of this analogue with cholesterol and other sterols. Most important, dehydroergosterol is a natural product and is present in significant quantities in eukaryotic organisms (Delseth et al., 1979; Sica et al., 1982) and is nontoxic to cultured cells (Rogers et al., 1979; Schroeder, 1981; Hale & Schroeder, 1982). In previous papers, we reported the use of dehydroergosterol for investigation of sterol–sterol interactions in palmitoyloleoylphosphatidylcholine SUV (Schroeder et al., 1987, 1988a,b; Nemecz et al., 1988; Nemecz & Schroeder, 1988). In summary, dehydroergosterol is an acceptable analogue for determining the preferential interaction of cholesterol with phospholipids.

The first experiments presented here were designed to evaluate whether cholesterol could interact preferentially with fluid or gel phases in phase-separated phosphatidylcholine mixtures. In 1:1 molar ratio phosphatidylcholine mixtures demonstrating gel-phase immiscibility, the fluorescence properties of dehydroergosterol indicated preferential interaction with fluid-phase phosphatidylcholine either in cosonicated SUV or in mixed SUV. In the mixed SUV, the dehydroergosterol exchanged between DSPC SUV and DMPC SUV with the rate being fastest when the acceptor SUV were in the liquid-crystalline phase. The latter observation is entirely consistent with data presented by Yeagle and Young (1986) using an assay requiring separation of donor and acceptor vesicles. Several additional observations may be made for the properties of dehydroergosterol in the DMPC/DSPC phase-immiscible pair:

(1) Although DSPC is the more rigid of the two phospholipids, the fluorescence polarization, limiting anisotropy, and order parameter of dehydroergosterol were lower in DSPC than in DMPC. One might have expected the lipid with the higher phase transition temperature, DSPC, to be more rigid and to increase the order sensed by the fluorescent sterol. This surprising finding can be rationalized as follows: (a) The dehydroergosterol may be so much less soluble in DSPC that at 0.5 mol % it may laterally phase-separate in the very rigid DSPC but not in the more fluid DMPC SUV or cosonicated SUV. Near 5 mol % sterol, cholesterol (Ipsen et al., 1987) and also fluorescent dehydroergosterol and cholestatrienol undergo lateral-phase separation (Schroeder et al., 1987, 1988a,b). Other investigators using freeze–fracture electron microscopy of cholesterol/phosphatidylcholine mixtures also observed microscopic phase separation of cholesterol from phospholipid below the phase transition temperature [reviewed in Houslay and Stanley (1982)]. As shown in phase-separated sphingomyelins (Figure 4) and PSP/DSPC (Figure 6), dehydroergosterol also demonstrated gel-phase immiscibility in the gel phase. (b) Alternately, the depth to which the fluorescent sterol is inserted into the very rigid DSPC membrane may differ considerably from its depth in more fluid lipids, and this may therefore alter the motional properties of the sterol itself. Results of other investigators indicate that as the rigidity of the phospholipid membrane increases, the polarity sensed by NBD-cholesterol, a fluorescent sterol analogue labeled with NBD in the alkyl side chain of cholesterol, increases dramatically (Kao et al., 1978). Elsewhere, we also reported that in POPC SUV the dehydroergosterol may reside in two domains, one of which is more polar than the other (Nemecz & Schroeder, 1988). Which, if not both, possibilities account for the observed data is not yet definitively known.

(2) Although cosonicated DMPC/DSPC SUV primarily display phase-separated gel phases, the fluorescent dehydroergosterol detected some gel–gel-phase miscibility of the DMPC/DSPC pair. Other investigators have reported that binary mixtures of phosphatidylcholines whose phase transition temperatures differ by less than 33 °C are completely or partially miscible in the gel phase (Curatolo et al., 1985; Mason, 1988). In contrast, when this difference in phase

transition temperatures is greater than 33 °C, gel-phase immiscibility is observed over a large concentration range. The difference in phase transition temperatures of the DMPC/DSPC pair is about 32 °C. Thus, the DMPC/DSPC pair is at the borderline between these trends, and partial miscibility of the gel phases might be expected.

(3) Fluid-phase immiscibility has not previously been reported to be a property of cosonicated phosphatidylcholine SUV [reviewed in Mason (1988)]. The data presented here for the cosonicated DMPC/DSPC SUV show for the first time evidence consistent with potential liquid-crystalline-phase immiscibility of phosphatidylcholines. Previous studies may not have observed this phenomenon because they utilized techniques that observed only "bulk" properties of membranes, i.e., DSC (Van Dijck et al., 1977; Matubayashi et al., 1986), neutron scattering (Knoll et al., 1981), and dilatometry (Schmidt & Knoll, 1986). In contrast, for those studies utilizing a probe approach (ESR), fluid–fluid immiscibility was also reported for binary mixtures of DMPC/cholesterol, DMPC/cardiolipin, and dielaidoylphosphatidylcholine/dipalmitoylphosphatidylethanolamine (Recktenwald & McConnell, 1981; Berclaz & McConnell, 1981; Wu & McConnell, 1975).

Although numerous studies have examined the behavior of binary mixtures of different phospholipids with identical acyl chains but different polar head groups [reviewed in Mason (1988)], much less is known of mixtures containing sphingomyelin. Herein, the preferential interaction of dehydroergosterol with sphingomyelin versus phosphatidylcholine was tested in several phase-separated sphingomyelin/phosphatidylcholine-cosonicated SUV systems: POPC/PSP, POPC/SSP, egg PC/brain SP, and PSP/DSPC. In the PSP/DSPC-cosonicated SUV, the PSP is the component with lower phase transition temperature while in the other mixtures the PC's are the lower phase transition temperature components. In all of these instances, the dehydroergosterol interacted preferentially with the lipid with the lower phase transition temperature whether it was sphingomyelin or phosphatidylcholine. The data indicated the presence of fluid–fluid-phase immiscibility of the two lipid species in cosonicated SUV at temperatures above either phase transition temperature. These observations are also consistent with those of other investigators using differential scanning calorimetry to show preferential association of cholesterol for phospholipid species with lower phase transition temperature in cosonicated SUV comprised of phase-separated mixtures including phosphatidylcholines, phosphatidylethanolamines, and phosphatidylserines (van Dijck et al., 1977; de Kruijff et al., 1973; Matubayashi et al., 1986; Knoll et al., 1981; Schmidt & Knoll, 1986). Thus, rather than being an exception to the rule, the sphingomyelins fit into the same pattern of sterol affinity as the other phospholipids. The present data agree with DSC results of others showing no preference of cholesterol for sphingomyelin in phase-miscible systems above the transition temperature (Untracht & Shipley, 1977; Calhoun & Shipley, 1979). Results of one cholesterol exchange experiment between membranes indicated that cholesterol had the same affinity for dipalmitoylphosphatidylcholine as for PSP at temperatures both above and below the gel to liquid-crystalline transition of these phospholipids (Lange et al., 1979). Since the transition temperatures of both these lipids were near 41 °C, these data are entirely consistent with those presented herein. The data presented herein, however, are in contrast to some obtained by differential scanning calorimetry indicating that sphingomyelin does not fit into the above series and that preferential interaction of cholesterol occurs with sphingomyelin (whether it is the lipid with the higher or lower phase transition temperature) (Demel et al., 1977). In addition, there is some evidence from experiments of cholesterol exchange between vesicles indicating that egg phosphatidylcholine had a lower affinity for cholesterol than did dipalmitoylphosphatidylcholine, PSP, or bovine brain SP (Lange et al., 1979). Likewise, bovine brain sphingomyelin dramatically slowed the kinetics of cholesterol transfer between POPC and sphingomyelin vesicles (Fugler et al., 1985; Yeagle & Young, 1986) and between vesicles and mycoplasma membranes (Clejan & Bittman, 1984). Reasons for the discrepancies are not known. However, several explanations may be offered. (a) In the differential scanning calorimetry experiments, 10–35 mol % of cholesterol was used in nearly all studies. Significant lateral phase separation of cholesterol occurs above 5 mol % (Schroeder et al., 1984a,b, 1987, 1988a,b; Rogers et al., 1979). Thus, depending on the temperature, areas of pure phosphatidylcholine, pure sphingomyelin, pure cholesterol, or various admixtures may be present at such high mole percent cholesterol. The preferential affinities of cholesterol within multiple phase-separated cholesterol and/or cholesterol/phospholipid complex pools are not understood. In the present work, only 0.5 mol % fluorescent sterol was used, a concentration well below that for lateral phase separation of sterol in fluid membranes (Schroeder et al., 1984a,b, 1987, 1988a,b). (b) The exchange and cosonicated SUV investigations may not be directly comparable. In exchange experiments, cholesterol must desorb from donor membrane, diffuse through the aqueous medium, and be taken up into acceptor membrane. The cholesterol taken up by the acceptor membrane must also subsequently equilibrate with several intramembrane cholesterol pools (Ipsen et al., 1987; Bar et al., 1988; Nemecz et al., 1988; Nemecz & Schroeder, 1988). In cosonicated SUV, most of these additional equilibrations are avoided. In any case, it is difficult to compare partition coefficients obtained from sterol exchange experiments between SUV to those obtained from intramembrane partitioning between phases within cosonicated SUV. (c) It is entirely possible that other factors including fatty acid composition, phase state, hydration, etc. may be important (Yeagle & Young, 1986). In fact, codistribution of cholesterol and sphingomyelin in the exofacial leaflet of biological membranes has not consistently been observed [reviewed in Schroeder and Nemecz (1989) and Kier et al. (1986)] and may even be highly dependent on the presence of polyunsaturated fatty acids (Sweet & Schroeder, 1988b). Incorporation of sphingomyelin into mycoplasma membranes also had no effect on the transbilayer cholesterol distribution (Clejan et al., 1981).

In summary, the data presented here are consistent with the conclusion that, at low mole percent, sterols preferentially interact as monomers with fluid-phase lipids in cosonicated SUV. A preferential interaction of monomeric sterol with sphingomyelin was not observed unless the sphingomyelin was the more fluid lipid.

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