Cholesteryl Sulfate-Phosphatidylcholine Interactions

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ABSTRACT

The effect of cholesteryl sulfate, a natural membrane component, on the physical state of dipalmitoyl phosphatidylcholine multilamellar vesicles was investigated using fluorescence polarization and differential scanning calorimetry techniques. Cholesteryl sulfate increased the order of acyl chains for those temperatures higher than the gel-to-liquid crystalline transition temperature while it decreased the order for those temperatures below the phase transition temperature. At equimolar concentrations, cholesteryl sulfate suppressed the crystal liquid-to-gel phase transition of dipalmitoyl phosphatidylcholine. These data suggest that sterol sulfates may provide new tools for the elucidation of molecular mechanisms involved in sterol-lipid interactions.

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INTRODUCTION

Cholesteryl sulfate (Chol So_4) is a membrane component that is widely distributed in nature and its isolation has been reported from both invertebrate (1) and mammalian tissues (2,3). Although it generally represents a small percentage of total membrane sterols, Chol SO_4 has a protective effect against osmotic shock of the erythrocyte membrane (3) and may be involved in membrane modifications of the spermatozoa (4). Researchers have also suggested that Chol SO_4 could be involved in ion transport (5). In contrast to the relationships between cholesterol and phospholipids (PL), which have been extensively studied (6-10), no data are available concerning the interaction of Chol SO_4 with PL.

In the present study we have demonstrated, using fluorescence polarization and differential scanning calorimetry techniques, that Chol SO_4 suppresses the liquid-gel phase transition of synthetic dipalmitoyl phosphatidylcholine.

MATERIALS AND METHODS

L-α-dipalmitoyl phosphatidylcholine (DPPC) was purchased from Sigma Chemical Co. (St. Louis, MO). Cholesteryl sulfate was synthesized and purified by chromatography as previously reported (11). Briefly, cholesterol was purified via the dibromide derivative and sulfurylated in dry pyridine and chlorosulfonic acid. Following partition chromatography on celite, the ammonium salt of the sterol sulfate was twice crystallized from aqueous methanol (m.p. 198-201 C) (12), and solutions were prepared in chloroform/methanol (1:2 V/V). Cholesterol (special grade) was obtained from Applied Science Lab. (State College, PA). 1,6-Diphenyl-1,3,5-hexatriene (DPH) was purchased from Aldrich Chemicals (Milwaukee, WI). The purity of all compounds was verified by thin layer chromatography (TLC).

Appropriate aliquots of stock solutions of DPPC and cholesterol in chloroform, or Chol SO_4 in chloroform/methanol, were mixed in test tubes. The solvent was removed under a stream of nitrogen and then under high vacuum overnight. Some samples were also freeze-dried to monitor the possible occurrence of a phase separation during the sample preparation. Both preparations provided identical results. For the fluorescence polarization experiments, DPH in a 2 mM stock solution in tetrahydrofuran was directly added to the mixtures before evaporation. The concentration of DPH was maintained constant at 1/1000 relative to the PL concentration. Lipid suspensions were prepared either in distilled water or in 50 mM Na phosphate buffer, pH 7.2, as previously described (13). Measurements were performed on a SLM 4000 apparatus equipped with a 4-cell thermostated compartment and a magnetic stirrer. A Nesslab temperature programmable circulatory bath was connected to the spectrofluorometer and the temperature was monitored with a thermolinear probe placed directly within the cell compartment. Light scattering was reduced to very low levels by the use of cut-off filters. In all conditions, the individual values obtained were the mean of at least 4 successive measurements which, by themselves, were the average of 10 determinations. The results of the steady-state depolarization experiments are expressed in terms of fluorescence anisotropy r, with $r = I\| - I\perp G / I\| + 2I\perp G$ where $I\|$ and $I\perp$ are the fluorescence intensity observed with the analyzing polarizer parallel and perpendicular to the polarized excitation beam. A correction factor, G, equal to $I'\perp / I'\|$, the primes indicating excitation polarized in a perpendicular direction, was used to correct for the unequal transmission of differently

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ABBREVIATIONS

DPH: 1,6-diphenyl-1,3,5-hexatriene; DPPC: L-α-dipalmitoylphosphatidylcholine; Chol SO_4: cholesteryl sulfate; Chol: choleserol.

polarized light. The lipid order parameter, S_{DPH}, was calculated according to the method of Jahnig (14).

Samples prepared for differential scanning calorimetry were hydrated with 80% water or Na phosphate buffer. These solutions were preheated to 10 C above the theoretical phase transition of the pure PL, transferred to standard aluminum sample pans and scanned at least twice at a rate of 5 C or 10 C/min using a DSC-1B Perkin-Elmer instrument. The extent of hydrolysis of the sterol sulfate during the course of the experiments was verified by the addition of ^{14}C-cholesteryl sulfate as internal standard. Following TLC and the assay of radioactivity, the degree of hydrolysis was found to be less than 1%.

RESULTS AND DISCUSSION

Because DPH does not partition strongly in favor of domains of different lipid composition or physical state (10), the measurement of its steady-state fluorescence anisotropy provides a rapid and sensitive index of the average of changes in the order of membrane lipids (14).

In lipid suspensions composed of DPPC-Chol SO_4 at various molar ratios, Chol SO_4 increased the anisotropy of DPH, i.e., the order of acyl chains for those temperatures higher than the gel-to-liquid crystalline phase transition temperature, T_m (Fig. 1). The reverse is true for temperatures below T_m. At equimolar concentration, Chol SO_4 suppressed the liquid-gel phase transition of DPPC.

Although cholesteryl sulfate, like cholesterol (6,8,10) decreased the order for $T < T_m$, increased the order for $T > T_m$ and suppressed the transition for molar ratios of 1:1, examination of the anisotropy vs temperature curves for different molar ratios (Fig. 2) revealed that the interactions in the Chol SO_4-DPPC systems were not identical to

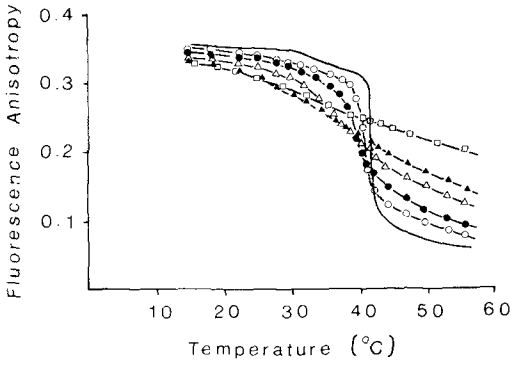

FIG. 1. Effect of increasing amounts of Chol SO_4 on the fluorescence anisotropy of DPH in DPPC suspensions. The solid line corresponds to pure DPPC. Molar concentrations of Chol SO_4 were (o) 5%, (●) 15%, (△) 25%, (▲) 33% and (□) 50%.

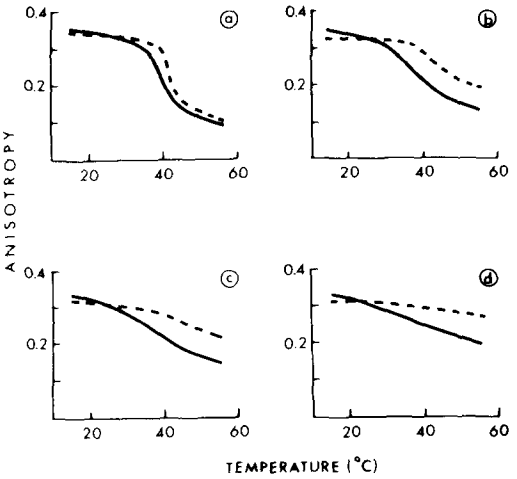

FIG. 2. Comparative effects of Chol SO_4 and cholesterol on the fluorescence anisotropy of DPH in DPPC suspensions. Molar concentrations of Chol SO_4 (full line) or cholesterol (dashed line): a: 15%, b: 25%, c: 33%, d: 50%. Note: the thickness of the lines are larger than the standard deviations of the determinations.

those found for the Chol-DPPC mixtures. Thus, the increase in order for $T > T_m$ was less pronounced than that observed with cholesterol. In particular, one does not observe the marked change that occurs between 15 - 25 mol % with cholesterol (Fig. 3a [10]). The increase in order obtained with Chol SO_4 exhibited an almost linear relationship to the fraction of the ester in the suspension. Using the same technique, other cholesterol esters, e.g., cholesteryl phosphorylcholine (15), cholesteryl hemisuccinate, cholesteryl betainate (16) and cholesteryl phosphate (17), have also been shown to increase, although to a lesser extent than cholesterol, the degree of order of egg PC liposomes or of lipids from the red blood cell. The use of DPPC enables us to demonstrate that for temperatures below the T_m, Chol SO_4 also decreased the order of acyl chains. However, for Chol SO_4/DPPC molar ratios higher than 15%, its effect was significantly more pronounced at 30 C than that of cholesterol (Figs. 2,3b) whereas the reverse was true at 20 C (Figs. 2,3c). Finally, Chol SO_4 caused a progressive shift in the transition temperature, which decreased from 41.6 C for pure DPPC to 40.4 C, 39 C, 36.3 C, and 35 C for Chol SO_4/DPPC molar ratios of 5%, 15%, 25% and 33%, respectively. Simultaneously, the transition broadened but remained detectable at 33% extending from 25 C to 45 C (Fig. 2c). The differential scanning calorimetry recording (Fig. 4) confirmed that, in contrast to cholesterol, for which very little change occurs in the midpoint temperature of the transition at increasing concentrations (6,9), Chol SO_4 produced a large downward shift in T_m. For both Chol SO_4-DPPC and

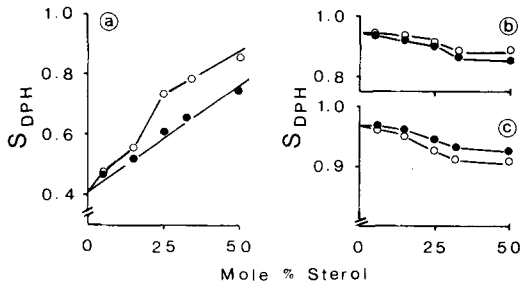

FIG. 3. Comparative effects of Chol SO_4 and cholesterol on the DPH lipid order S_{DPH}. Temperatures: 50 C (3a), 30 C (3b) and 20 C (3c); (●): Chol SO_4, (o) cholesterol.

Chol-DPPC (in control experiments) no further phase transition was detected when the relative concentration of the sterol attained 33 mol%. This value agrees with that observed by most investigators using similar equipment where detecting the presence of broad peaks is difficult (9).

The approximately linear relationship between the Chol SO_4 content of the vesicles and the decrease in transition temperature as well as the broadening of the transition suggests that part of the effects of Chol SO_4 may be caused by nonspecific interactions. In accord with classical thermodynamic theory (18), small molecules, e.g., anesthetics (19) and alcohols (20), can broaden as well as lower the transition temperature of a pure lipid. On the other hand, this factor would not account for both the fluidizing ($T < T_m$) and rigidifying ($T > T_m$) effects of Chol SO_4. The fact that equivalent amounts of cholesterol, cholesteryl sulfate and 5-α-cholestan-3-one (20,21) are required to suppress completely the gel-liquid transition would also support the importance of the sterol nucleus and of the Van der Waal's force between this nucleus and the acyl chains in this suppression. This agrees with the recent work of Bittman et al. (22) indicating that no specific or direct interaction of the PC head group with cholesterol occurs.

In conclusion, these studies demonstrated that cholesteryl sulfate, a naturally occurring cholesteryl ester, can suppress the liquid-gel transition of DPPC. It also increased the order of lipids for temperatures above the transition temperature and decreased this order for temperatures below. Although further investigation is indicated to clarify the nature of the interaction between cholesteryl sulfate and DPPC, our results suggest that sterol sulfates may represent new tools for the elucidation of molecular mechanisms involved in sterol-lipid interactions.

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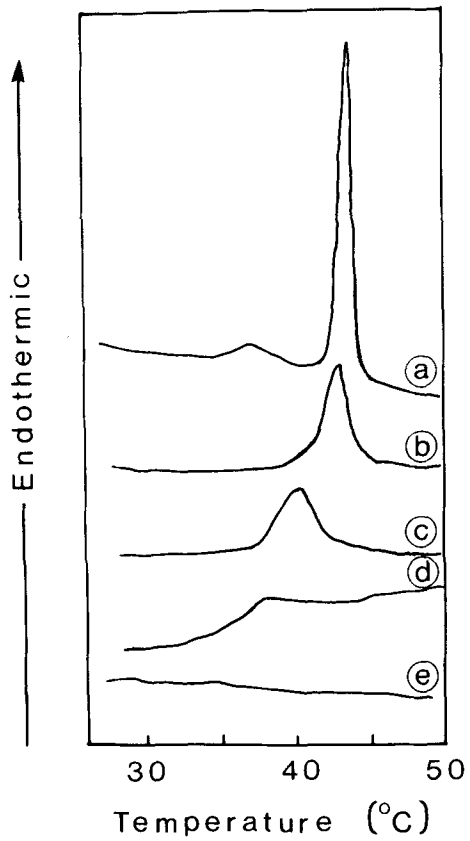

FIG. 4. DSC recordings of the effects of increasing amounts of Chol SO_4 on the transition of DPPC (heating scans). Heating rate 5 C/min. Molar concentration of Chol SO_4 (%): a: 0.0, b: 5.0, c: 13, d: 25, e: 33.

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