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EFFECT OF STEROL DERIVATIVES ON NATURAL PHOSPHOLIPID WITHIN LIPID BILAYER MEMBRANE

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ABSTRACT

This study investigated the physicochemical characteristics of vesicles that were prepared from two phosphatidylcholines and three sterol derivatives (SD) with different hydrophilic head groups, by measuring particle size and zeta potential. The aim was to further investigate the interaction between saturated and unsaturated phosphatidylcholine mixtures of SD and their storage stability. Results obtained from dynamic light scattering (DLS), zeta potential and transmission electron microscopy (TEM) show that spherical and/or angular vesicles of phosphatidylcholine/SD can be formed by self-assembly in aqueous solutions. The experimental data reveal that the incorporation of SD into lipid vesicles causes significant changes in the size, zeta potential, storage stability, and membrane fluidity of colloids, which may be correlated with the structure of the SD, the degree of saturation of the lipids, and the vesicular composition. The phosphatidylcholine/NaDC vesicles showed a smaller particle size and higher negative zeta potential. Furthermore, characteristics of vesicles dispersed in water were studied by differential scanning calorimetry (DSC) and fluorescence polarization. The phosphatidylcholine/NaDC vesicles showed a lower main transition temperature, enhanced membrane fluidity, and better storage stability. The information obtained from these studies may be useful for the design of novel drug carrier systems.

Keywords: Sterol derivatives; sodium deoxycholate; cholesterol; fluorescence polarization; differential scanning calorimetry.

1. INTRODUCTION

Sterols play an important role in membrane components and are implicated in the regulation of properties such as permeability and fluidity (Finegold *et al*. 1992; Bloom and Mouritsen 1988; Bloch e*t al*. 1983). A considerable amount of research has been devoted to sterols, such as cholesterol, to understand the physical basis of their biological functions in cells by investigating their role in modulating the physical properties of artificial and biological membranes, and to unravel the relationship between their functions and molecular structure (Yeagle *et al*. 1991; Razin and Rottem 1978; Ohvo-Rekilä e*t al*. 2002; McMullen e*t al*. 1999). An intriguing question, the question of the origin of cholesterol in the context of cell evolution, remains unanswered. One approach in determining the biological role of sterols in membranes is to examine the structural specificity of various sterols in different configurations. This approach was also used in this study.

It has been reported that cholesterol shows a condensing effect on the molecular arrangement as phospholipids in the liquid-crystalline state. This may be due to the rigid structure of the sterol reducing the possibility for cis-trans isomerizations between side chains of lipids. But a disordering effect on the membrane packing was found as liposomes below the chain-melting transition temperature, where the lipid bilayer was in the gel state (Mouritsen 1991; Lipowsky 1995; Demel and Kruyff 1976; Yeagle 1985; Finean 1990; Dahl 1981; Yamauchi *et al*. 1993; McMullen *et al*. 1995; McMullen and McElhaney 1996; Slotte 1995; Reis *et al*. 1996). Nevertheless, the degree of these effects was related to the molecular structures of the phospholipids as well as the sterols (Bernsdorff and Winter 2003; Endress *et al*. 2002; Martinez *et al*. 2004; Henriksen *et al*. 2004). The factors controlling the physical state of natural membranes at a molecular level are difficult to understand due to their structural complexity. To gain information about specific lipid-sterol interactions, realization of simple, well-defined model biomembrane systems is necessary. In the liquid-ordered phase of phospholipid, the acyl chains are extended and relatively tightly packed (Vist *et al*. 1990; Ipsen *et al*. 1987; Thewalt *et al*. 1992), but there is still a relatively high degree of translational motion in the plane of the bilayer (Silvius 2003; Martin *et al*. 2005). This work used hydrogenated soy phosphatidylcholine (HSPC) to form liposomes as a membrane model for realization interaction of HSPC with three sterol derivatives (SD) including sterol oxime (SO), sodium deoxycholate (NaDC), and cholesterol (Chol).

For the first, SO was synthesized successfully in our laboratory. The hydrophilic head group was modified with hydroxylamine, a main component of cholesteryl chloroformate. Besides, bile salts are physiological surfactants that play an important role in the intestinal digestion and absorption of dietary lipids and cholesterol. These surfactants solubilize lipid vesicles by transforming them into mixed micelles (Subuddhi and Mishra 2007).

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Bile salts have a rigid planar hydrophobic moiety consisting of a steroid nucleus with two or three hydroxyl groups and are a special group of biosurfactants with properties that differ from ordinary aliphatic surfactant molecules (Madenci and Egelhaaf 2010). They solubilize and emulsify cholesterol and lipids in the intestine, respectively. In addition, the aggregation properties of bile salts are completely different compared with normal detergents due to their structure and rigidity (Madenci and Egelhaaf 2010). Among the most frequently studied are sodium cholate (NaC) and sodium deoxycholate (NaDC) (Ramos *et al*. 1999). Sodium deoxycholate (NaDC) is a secondary bile salt which is formed from primary sodium cholate by a bacterial enzyme in the upper small intestine. It was used in the preparation of mixed phospholipid/sterol derivative vesicle systems in this study.

Many studies have explored the effects of sterol derivatives in phospholipid membranes with regards to phospholipid condensation, membrane permeability to small solutes (Yamauchi *et al*. 1993; Demel *et al*. 1972), phospholipid order (Schuler *et al*. 1990; Schuler *et al*. 1991; Mel'nikov *et al*. 2004), membrane interface quality (Silva *et al*. 2011), thermotropic properties of phospholipid bilayers (Xu *et al*. 2001), and membrane domains (Chang *et al*. 2012). Systems consisting of lipid/sterol derivatives/water typically show a variety of structures (lamellar, vesicles, worm-like micelles, globular micelles, and rod-like micelles) that vary according to the relative amounts of the three components. The purpose of this study was to determine whether different sterol derivatives and phospholipids could modify the physical structure in the lipid bilayer and cause changes in its structure, fluidity, and thermodynamic properties. The focus of this study was to identify and characterize the differential effects of various sterol derivatives, including sterol oxime (SO), sodium deoxycholate (NaDC), and cholesterol (Chol), which is considered the evolutionary precursor of sterol derivatives (shown in scheme 1), on the equilibrium structure of the lipid bilayer membrane and relate those effects to the differences in their molecular chemistry.

The molecular structure of SO was verified by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy and Fourier transform infrared (FTIR) spectroscopy. Furthermore, various methods were applied to study the aggregation behavior of mixed lipid/sterol derivative systems and vesicle transition, including transmission electron microscopy (TEM), dynamic light scattering (DLS), fluorescence polarization, and differential scanning calorimetry. The results provide valuable information for further applications of mixed lipid/sterol derivative vesicles as possible candidates in drug/biomimetic membrane systems. Differential scanning calorimetry (DSC) and fluorescence polarization (FP) were applied to investigate the effects of molecular interactions between sterol derivatives and phosphatidylcholine in the lipid bilayer vesicles (liposomes), which could also be used as a cell membrane model. DSC analysis showed that the incorporation of sterol derivatives into the HSPC bilayer caused a reduction in the cooperativity of the bilayer phase transition and led to a looser and more flexible bilayer structure. Thus, including sterol components in the HSPC/sterol derivatives mixed bilayer could facilitate molecular interaction between sterol derivatives and lipid and make the system more stable. These results provide valuable information for further applications of mixed lipid/sterol derivative vesicles as possible candidates in drug/biomimetic membrane systems.

2. MATERIALS AND METHODS

2.1 Materials

Hydrogenated soy phosphatidylcholine (HSPC, purity 99%) and egg phosphatidylcholine (EPC, purity 99%) were purchased from Nippon Oil and Fats Co., Ltd. (Kanagawa, Japan). Cholesteryl chloroformate (purity 97%) and cholesterol (Chol, purity 99%) were purchased from Sigma-Aldrich Co. (USA). Sodium deoxycholate (NaDC, purity 98%), and hydroxylamine (50% aq. soln.) were purchased from Alfa Aesar (UK). The fluorescent probe 1, 6-diphenyl-1, 3, 5-hexatriene (DPH) was purchased from Sigma-Aldrich Co. (USA). All the chemicals were used as received without further purification. All the solvents used in the syntheses were purified, dried, or freshly distilled as required. Pure water with a resistivity of 18.2 MΩ cm was obtained from a Milli-Q plus purification system (Millipore).

2.2 Preparation of lipid/SD vesicles

A mixture of phosphatidylcholine and SD under a defined molar ratio was weighed and dissolved into a mixed organic solvent of chloroform/methanol with a volume ratio of 1:1. The organic solvent was slowly removed through the reduction of vapor pressure with a rotary evaporator at 50 °C for 1 h which resulted in a thin, dry film on the wall of the round-bottomed flask. The thin, mixed film of phosphatidylcholine and SD was dried for a further 30 min under vacuum to completely remove any residual organic solvent. Then, the thin, dry film was hydrated with pure water for 30 min at 50 °C to obtain multilamellar vesicles. Once the hydration process was completed, a high performance ultrasonic machine (Sonicator 3000, Misonix Incorporated) was used to sonicate the vesicles at 50 °C with a power of 90 W to reduce their size. The machine came equipped with a cup of corn to avoid samples making direct contact with the titanium probe. The total concentration of each mixed phosphatidylcholine and SD vesicle system was fixed at 5 mM.

2.3 Transmission electron microscopy

Micrographs of vesicles were carried out by TEM (model JEM-1400, JEOL Ltd., Japan). Drop coating was used to apply the sample to a carbon-coated copper grid (200 mesh) and then this was air-dried for 2 min. After removal of the excess sample, uranyl acetate (1% w/v) solution was added. The sample was air-dried overnight and then observed at 120 kV.

2.4 Size and zeta potential determination

The size and zeta potential distributions of dispersions were examined at 25 °C using a DLS instrument (90Plus with a Zeta Potential Analyzer, Brookhaven Instruments Corporation, USA) equipped with a 570 nm laser and an optical detector with a scatter angle of 90°. The particle size, referred to as the hydrodynamic diameter (d), was calculated from DLS data using the Stokes–Einstein equation ($d = {}_{kB}T/3_{\pi\eta}D$), where *kB* is the Boltzmann constant, T is the absolute temperature, η is the solution viscosity, and D is the diffusion coefficient. The zeta potential of dispersions can also be determined by measuring electrophoretic mobility with the Smoluchowski equation.

2.5 Fluorescence polarization

Vesicles were labeled with a lipophilic fluorescent molecule, DPH. Aliquots of DPH dissolved in a mixed chloroform/methanol (1/1, v/v) solvent were added into the mixed stock solution, and then the vesicles were prepared as described above. DPH was embedded in the lipid bilayer of vesicles, and the molar ratio of DPH:lipid was 1:1000. The steady-state fluorescence polarization measurements were performed at 25 °C using an LS 55 Fluorescence Spectrometer (PerkinElmer). The excitation wavelength was set at 360 nm, and the emission intensity was monitored at 450 nm. The fluorescence polarization values of the vesicle samples were calculated using the equation (Chou *et al*. 2014):

$$P = \frac{I_{par} - GI_{per}}{I_{par} - GI_{per}}$$

where Ipar and Iper indicate the fluorescence intensity with polarization parallel and perpendicular to the excitation beam polarization, respectively, and G = 0.87 is the instrumental correction factor for optical variations between the parallel and perpendicular emission measurement paths. All experiments were done with multiple sets of samples, and the average values of polarization were reported.

2.6 Differential scanning calorimetry

DSC measurements were performed using a Mettler Toledo (Schwerzenbach, Switzerland) instrument with empty hermetically sealed aluminum pans as the reference. Data were processed using SigmaPlot software. The concentration was 20 mM, and 20 µl of liposome suspension was carefully placed and sealed in the aluminum hermetic pans. The samples were examined at a scanning rate of 5 °C/min by applying a heating and cooling cycle from 10 °C to 80 °C, which was then repeated three times (Chou *et al*. 2014).

3. RESULTS AND DISCUSSION

3.1 Physicochemical characteristics and physical stability of lipid/ SD vesicles

The morphology of the vesicles was observed using TEM. An electron micrograph of mixed 9/1 HSPC/SD vesicles dispersed in water is presented in Fig. 1. TEM images (Fig. 1 (A)) show that HSPC/SO vesicles were almost spherical in shape and approximately 410 nm in size, which was slightly smaller than the size determined by DLS. A transmission electron microscope was used to examine the morphology of vesicles composed of 90 mol % HSPC and 10 mol % SO. The results showed the presence of spherical or angular vesicles with a diameter of < 100 nm and disrupted vesicles in the bilayer structure. TEM images (Fig. 1 (B)) show that HSPC/NaDC vesicles were nearly spherical and angular in shape and approximately 190 nm in size, which was slightly smaller than the size determined by DLS. This was mainly due to the process involved in the preparation of the sample. TEM depicted the size of the sample in a dried state, whereas DLS determined the size in a hydrated state. Therefore, the size determined by DLS was a hydrodynamic diameter and was larger than the size measured by TEM due to solvent effect. The mixed HSPC/Chol systems yielded similar results, indicating that the incorporation of Chol into HSPC liposomes with a molar fraction of 10 % changed the vesicle structure. The morphology of the vesicles was observed using TEM. An electron micrograph of mixed 9/1 (HSPC/Chol) vesicles dispersed in water revealed a slightly angular morphology and relatively large particles, as presented in Fig. 1 (C). This phenomenon indicates that the subsequent ultrasonication step had a great effect on the physicochemical characteristics of the mixed HSPC/SD system. Size distribution and zeta potential measurements are often used to determine vesicle characteristics in aqueous dispersions. Pure HSPC vesicle dispersions showed a smaller average vesicle size and neutral potential. The average particle size and zeta potential of mixed phosphatidylcholine/SD vesicles in water solution were also measured and Fig. 1 (D), Fig. 1 (E), and Fig. S2 (A) and Fig. S3 (A) of the supplementary information, present the results, respectively. The mixed HSPC/SO vesicles had a larger average particle size, a lower zeta potential, and poorer stability at room temperature than the other two mixed systems (Fig. 2), especially at a molar ratio of HSPC/SO = 10%. Particle size increased significantly as the molar fraction of SO increased up to 50%. Higher molar fractions of SO or Chol (10% and 50%) in the HSPC vesicle were associated with a smaller mean particle size and a higher negative zeta potential (Fig. S2 (A)). The morphological stability of these three systems followed the order: mixed HSPC/NaDC > HSPC/Chol > HSPC/SO vesicles, as determined from the average particle size, mean zeta potential, polydispersity index, and stability, as listed in Fig. 1 (D), Fig. 2, and Fig. S1 (B) and Fig. S2 (B) of the supplementary information, respectively. These results show that NaDC, with a more hydrophilic head group, may be more effectively incorporated or packed into HSPC vesicular membranes. Furthermore, a net negative charge was observed for all the mixed HSPC/SO vesicle formulations (Fig. S2 (A) of the supplementary information). As SO was incorporated into the lipid vesicles (SO = 10%), the zeta potential of the vesicles significantly decreased and approached the value of approximately -15-20 mV. This may be due to the fact that SO head groups have a negative charge and, therefore, quickly saturated the surface charge density of the vesicles.

Fig. 1 (E), Fig. S1, and Fig. S2 present the average particle size, polydispersity index and zeta potential of EPC into which SO, NaDC, and Chol were incorporated in the water solution at room temperature. Adding 10-50 mol % SO seemed to increase the size of the EPC liposomes. A larger average particle size of vesicles was observed when the vesicles incorporated lower molar fractions of SO. The zeta potential analysis of EPC/SO formulations indicated that the vesicles had a net negative charge (approximately -20-40 mV) due to the addition of SO with negatively charged head groups. This stabilizing effect presumably followed from the repulsive interactions among vesicles with negative surface charges which inhibited the fusion or aggregation of vesicles. The EPC dispersions that incorporated 10-50 mol % NaDC had a smaller particle size and better storage stability than the SO and Chol dispersions (Fig. 2 (B)). The most stable formulation, as determined by size, varied with time and occurred at the molar ratio of EPC/NaDC = 9/1 as shown in Fig. 2 (B). TEM images show that EPC vesicles with 10 mol % of NaDC exhibited a smoother outline (Fig. 1 (B)) than other dispersions (Fig. 1 (A) and (C)), which may explain the better storage stability of the dispersion. In the mixed EPC/Chol system, the diameter of the EPC dispersions increased with the addition of 10 ~ 50 mol % of Chol (Fig. (C)). This may be due to the fact that Chol has a tetracyclic steroid ring system which may have led to steric hindrance.

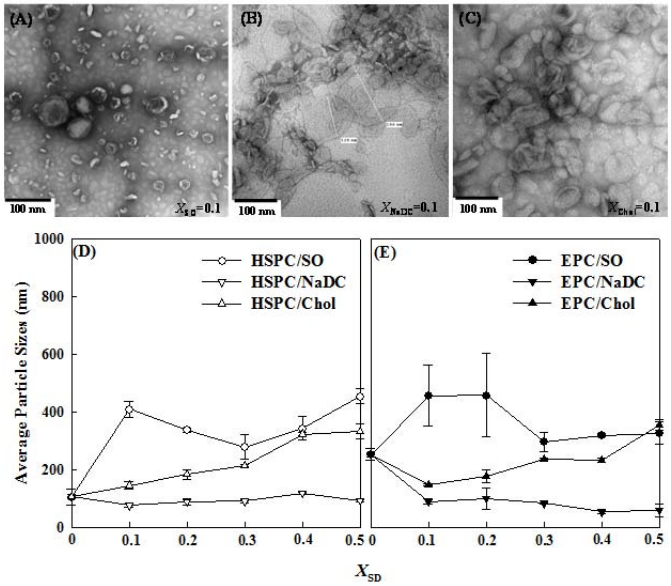


Fig. 1 TEM images of HSPC vesicles with 0.1 molar fraction, namely (A) SO, (B) NaDC and (C) Chol of sterol derivatives. The total lipid concentration in the vesicular dispersion is fixed at 5 mM. Average particle sizes of (D) HSPC and (E) EPC vesicles with molar fraction of sterol derivative (X_{SD}) at 24.5 °C. Data represent the mean ±standard deviation of three independent measurements.

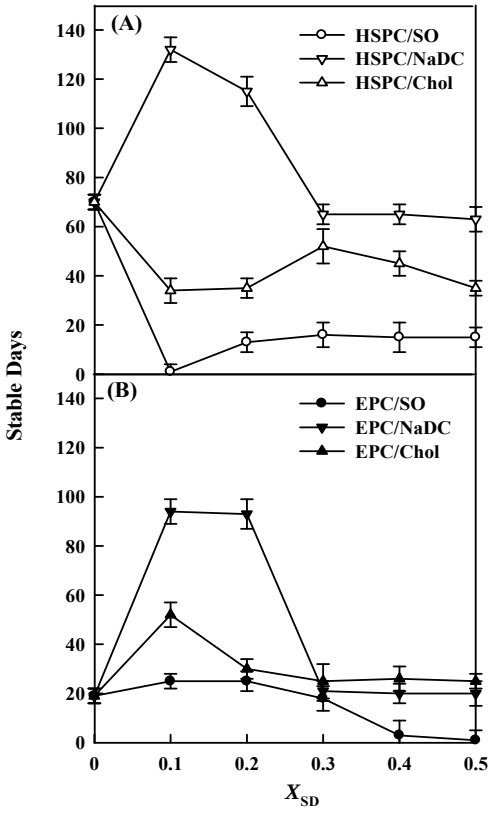


Fig. 2 Stable days of (A) HSPC and (B) EPC vesicles with various molar fractions of steroid derivatives (X_{SD}) at 24.5 °C. The criterion of vesicle stability is determined by APS < 500 nm and/or PDI < 0.4. Data represent the mean ±standard deviation of three independent measurements.

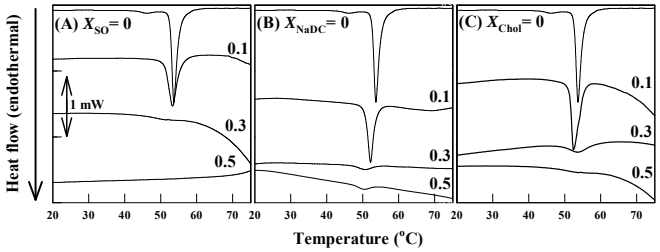


Fig. 3 DSC thermograms of HSPC vesicles with various molar fractions (A) SO, (B) NaDC and (C) Chol. The total lipid concentration for each sample is fixed at 20 mM.

3.2 Differential scanning calorimetry assays

The membrane phase behavior of mixed HSPC/SD vesicles with various molar fractions of SD (X_{SD}) was monitored by DSC, and the resulting thermograms are shown in Fig. 3. In addition, the thermodynamic characteristics, including the main transition temperature (T_m), melting enthalpy change (ΔH), entropy change (ΔS), and peak width at half height ($\Delta T_{1/2}$), which were obtained from Fig. 3, are summarized in Table 1. The endothermal curve of pure HSPC vesicles exhibited only a single narrow peak due to the transition from a gel phase to a liquid crystalline phase. The peak position at 53.57 °C gave the T_m of HSPC vesicles at a concentration of 20 mM. Fig. 3 (A) reveals that a small amount (*i.e.*, X_{SO} = 0.1) of SO incorporated into HSPC vesicles resulted in a decrease in the values of T_m, ΔH and ΔS. This implies that adding a small amount of SO could disturb the close packing of pure HSPC membranes. As X_{SO} increased to 0.3, a single broad peak with a reduced ΔH and ΔS at 53.24 °C was observed, which suggested that a better degree of molecular mixing with a looser packing occurred compared to that of pure HSPC vesicles. As X_{SO} further increased to 0.5, the temperature of the main transition disappeared. These results show that the phase transition became broader and then vanished completely with an increased concentration of SO, as seen in Fig. 3 (A). However, in mixed HSPC/NaDC systems, DSC heating scans of HSPC dispersions contained different concentrations of NaDC and are shown in Fig. 3 (B) for comparative purposes. The overall pattern of behaviour seen on heating was that increasing the NaDC concentration reduced the temperature, enthalpy and entropy while decreasing cooperativity in both HSPC/NaDC mixtures. As shown in Fig. 3 (B), considerable broadening on the peaks occurred in the mixed HSPC/NaDC system which increased the $\Delta T_{1/2}$ values (Table 1), and showed evidence of a decrease in phase transition cooperativity. Furthermore, the peak temperature decreased slightly, but enthalpy and entropy increased slightly with a higher NaDC concentration (X_{NaDC} = 0.5). This was possibly related to the NaDC molecular structure - the smaller enthalpies of transfer of solutes from a gel to a fluid phase could be the result of destabilization of the gel phase relative to the fluid phase of the pure phospholipid. Thus, it could be suggested that NaDC positions itself with the carboxylic group interacting with the head group of HSPC and the steroidal nucleus parallel to the acyl chains at the first few carbons of these chains. It is noted that cholesterol

Table 1 Main-transition temperatures (T_m), enthalpy change (ΔH_m), enthropy change (ΔS), and peak at half-height ($\Delta T_{1/2}$) for various molar fractions of sterol derivatives (X_{so}, X_{NaDC} and X_{Chol}) obtained from Fig. 3.

Molar fractions	T_m (°C)	ΔH_m (kJ · mol^{-1})	ΔS (J/mol · K)	$\Delta T_{1/2}$ (°C)
X_{SO}				
0	53.57	51.58	158.26	1.61
0.1	53.82	17.51	53.58	2.80
0.3	53.24	1.80	5.51	6.14
0.5	*	*	*	*
X_{NaDC}				
0	53.57	51.58	158.26	1.61
0.1	51.69	28.93	89.06	1.76
0.3	50.22	1.92	5.94	3.97
0.5	50.05	2.21	6.83	4.51
X_{chol}				
0	53.57	51.68	158.26	1.61
0.1	52.49	34.43	105.52	2.15
0.3	53.60	2.91	8.91	5.53
0.5	*	*	*	*

*Indicated that data were not monitored.

and lipid clustering in membranes can be differentiated from HSPC/Chol constituted membranes. Regarding the main transi tion, both the enthalpy and entropy of the transition decreased in a linear manner with increasing Chol concentration and the main phase transition completely disappeared above 50 mol % Chol. The enthalpy and entropy of the overall main phase transition decreased with an increase in Chol concentration (see Fig. 3 (C)). This suggests that the mixed HSPC/Chol vesicles exhibited less cooperativity in the main transition compared with vesicles composed of pure HSPC. Thus, the Chol was inserted into the membrane with its hydroxyl group turned toward the aqueous surface and the aliphatic side chain positioned parallel to the acyl chains of the phospholipid. The 3β-hydroxyl group was aligned with the carboxyl residues of the phospholipid ester linkages, which led to limited freedom of vertical movement.

3.3 Membrane fluidity of vesicles in the gel phase

Fluorescence polarization is a commonly used method to evaluate the molecular packing order of lipid membranes. DPH has been widely used to evaluate the membrane fluidity of various kinds of LUVs and when incorporated into the deeper lipid bilayer can provide information about the molecular arrangement of the hydrophobic regions because the probe has a greater rotational degree of freedom in the loosely packed state of the lipids. Fig. 4 (A) illustrates the degree of fluorescence polarization of mixed lipid/SD vesicles as a function composition at room temperature. In the mixed HSPC/SO systems, a slight decrease in the polarization values of the HSPC vesicle occurred at low molar ratios of SO (10%) and a sudden decrease occurred when the composition of SO ≥ 20 mol %. This implies that the incorporation of a small amount of SO into the HSPC vesicles led to a more disordered molecular packing of the lipid bilayer domain as well as a slight decrease in membrane fluidity. However, SO ≥ 20 mol % could have a significant effect on electrostatic repulsion, and polar head group interactions may indirectly facilitate the molecular fluidity of vesicle membranes. Furthermore, in the mixed HSPC/NaDC systems, the polarization values of the HSPC vesicles noticeably increased at molar ratios of NaDC ≥ 20 mol %. A high degree of polarization reflects a limited rotational diffusion of the probes and, therefore, represents a high structural order or low membrane fluidity. These results indicate that a vesicular bilayer with this composition could exhibit a higher-order molecular arrangement and a more rigid membrane. In the mixed HSPC/Chol systems, the polarization values of the HSPC vesicles decreased slightly at low molar ratios of Chol (10%) and increased slightly when the composition of Chol ≥ 20 mol %. This suggests that the incorporation of a small amount of Chol into HSPC vesicles led to a less disordered molecular packing of the lipid bilayer domain as well as a slight decrease in membrane fluidity. However, Chol ≥ 20 mol % provided a significant hydrophobic-chain interaction which led to a more ordered lipid bilayer domain and a slight decrease in membrane fluidity.

In Fig. 4 (B), the polarization values of pure EPC vesicles are shown as approximately 0.12 P, which is lower than that of pure HSPC (~ 0.39 P). Pure EPC vesicles (main phase transition temperature, T_m ~ −5 °C) were in a liquid-crystalline state at 24.5 °C, while pure HSPC vesicles (T_m ~ 53 °C) were in a gel state. Regarding molecular structure, EPC has an unsaturated hydrocarbon chain which results in an increase in acyl chain mobility. In the mixed EPC/SO systems, a maximum polarization value appeared at SO = 30 mol %, which indicated that the vesicular bilayer with this composition exhibited the highest order in terms of molecular arrangement and had the most rigid membrane. The interaction of SO with lipid polar head groups increased the rigidity of the membrane. Thus, the evidence shows that the molecular fluidity of vesicle membranes decreased with SO from 0 to 30 mol % and then increased with SO from 30 to 50 mol %. Furthermore, in the EPC dispersions, the fluorescence polarization value of DPH increased significantly as the molar fraction of NaDC increased from 0 to 10 mol %, and increased slightly as the molar fraction of NaDC increased from 20 to 50 mol %. Although there was not a significant difference in the fluorescence polarization of the various compositions, the addition of 20 ~ 50 mol % NaDC elevated the rigidity of the pure EPC membrane slightly. In the mixed EPC/Chol systems, the membrane fluidity of EPC dispersions decreased significantly as the Chol mol % increased from zero to 10%, but increased for > 20 mol % Chol. EPC dispersions with 20 mol % Chol displayed the maximum magnitude of polarization, which implies that this composition exhibited the highest order of molecular arrangement compared with those of the other compositions. Chol exhibited a more significant disturbance on the membrane arrangement of EPC dispersions than SO or NaDC, which was possibly due to its very rigid, flat molecular shape disrupting the packing order of the neighboring lipids.

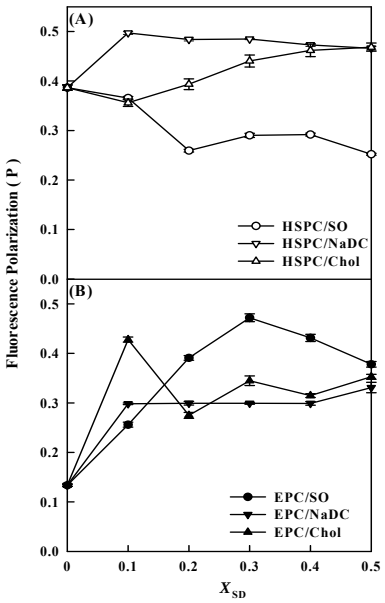
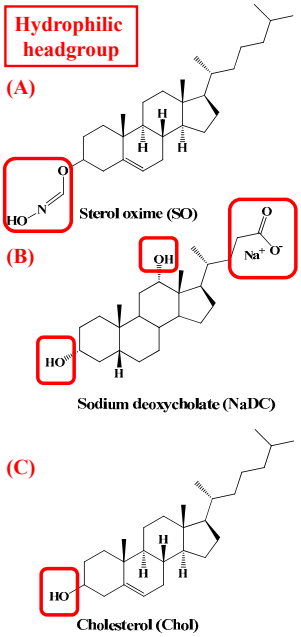


Fig. 4 Fluorescence polarization of (A) HSPC and (B) EPC vesicles with the molar function of the sterol derivatives at 24.5 °C. Data represent the mean ±standard deviation of three independent measurements.



Scheme 1 Chemical structures of sterol derivatives (SD), namely (A) sterol oxime (SO), (B) sodium deoxycholate (NaDC) and (C) cholesterol (Chol).

4. CONCLUSION

This study showed that the addition of various SD into lipid vesicles significantly changed the physicochemical properties of dispersions. These changes depended on the molar ratio of the SD in the vesicles, the categories of lipids, and the different SD polar head groups. Incorporating 10 ~ 50 mol% SO into PC vesicles resulted in larger particles, higher negative zeta potential, lower polydispersity, and less storage stability than pure lipid vesicles. The freedom of molecular motion in the HSPC bilayer due to the addition of SO was more remarkable than in the EPC bilayer. The lipid/SO vesicles exhibited a more significant disturbance in the membrane arrangement of lipid dispersions than the other SD did. In addition, the lipid/NaDC vesicles showed smaller particle size, higher negative zeta potential, more spatial structure, a lower main transition peak, and better storage stability than the lipid/SO vesicles. The evidence suggests that NaDC has a steroidal backbone similar to that of the membrane stabiliser cholesterol and that the presence of the carboxylate and hydroxyl groups in NaDC produce an activating effect on the edge rather than a membrane stabilising effect. With fully hydrated PC bilayers, interfacial water is the only source of donor groups available for hydrogen-bonding to the carbonyl groups and, as a result, assignment of the H-bonded population of carbonyls can be relatively straightforward and unambiguous. These results suggest that mixed lipid/SD vesicles have good potential for drug delivery, especially for lipid/NaDC vesicles with $X_{NaDC} = 0.1$, which exhibit the best storage stability.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY DATA

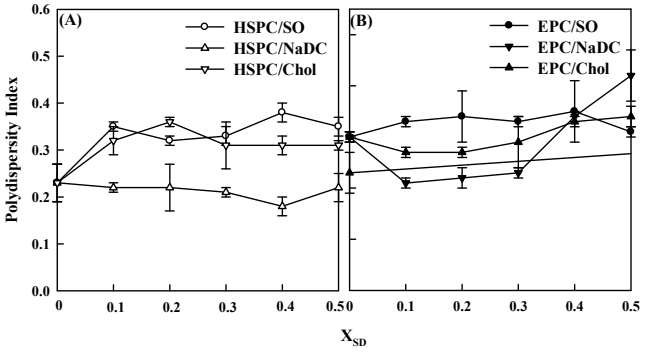

Fig. S1. Polydispersity index (PDI) of (A) HSPC and (B) EPC vesicles with the molar function of the sterol derivatives at 24.5 °C. Each datum is mean ±standard deviation from three independent measurements.

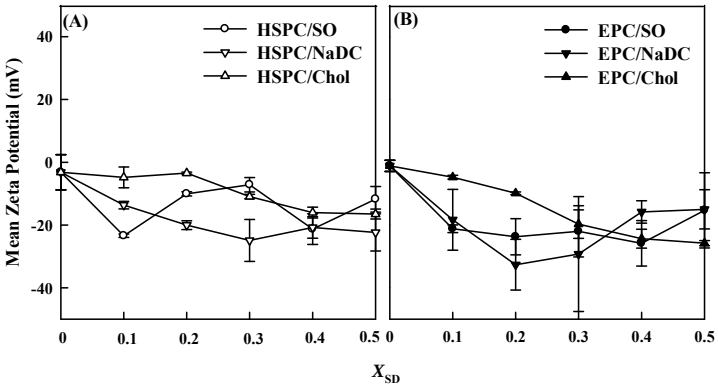

Fig. S2. Zeta potential of (A) HSPC and (B) EPC vesicles with the molar function of the sterol derivatives at 24.5 °C. Each datum is mean ±standard deviation from three independent measurements.

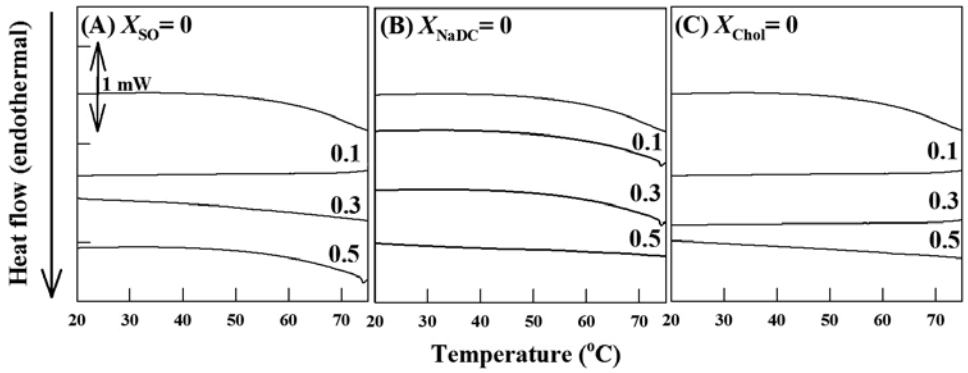

Fig. S3. DSC thermograms of EPC vesicles added with various molar fractions of (A) SO, (B) NaDC and (C) Chol. The total lipid concentration for each sample is fixed at 20 mM.

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