Colloids and Surfaces B: Biointerfaces 161 (2018) 59–66

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Full length article

Effects of sodium β-sitosteryl sulfate on the phase behavior of dipalmitoylphosphatidylcholine

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ARTICLE INFO

Article history:
Received 2 August 2017
Received in revised form
27 September 2017
Accepted 3 October 2017
Available online 6 October 2017

Keywords:
Phosphatidylcholine
β-sitosteryl sulfate
Phase
SAXS
DSC

ABSTRACT

We have studied the phase behavior of dipalmitoylphosphatidylcholine (DPPC) containing sodium β-sitosteryl sulfate (PSO_4). PSO_4 was found to lower the phase transition temperature of DPPC to a higher degree than cholesterol or β-sitosterol. It also gave rise to the formation of a modulated (ripple) phase (P_β) at low to moderate concentrations. At concentrations greater than 25 mol%, it completely changed the membrane into a fluid phase. This shows that PSO_4 is capable of disordering the hydrocarbon chains of PC efficiently. The characteristics of PSO_4 for fluidizing the membrane can be useful for the pharmaceutical and cosmetics industries.

1. Introduction

Phosphatidylcholines and sterols are two important classes of membrane constituents [1–5]. Phosphatidylcholines such as DPPC (dipalmitoylphosphatidylcholine), DMPC (dimyristoylphosphatidylcholine), DSPC (distearoylphosphatidylcholine), etc. are amphiphilic molecules that arrange themselves into lamellar bilayers in the presence of water. Such membranes exhibit thermotropic and lyotropic phase behaviors that are of great biological significance. The three major phases that these phospholipids assume are a gel phase (L_β') at low temperatures, a liquid crystalline (L_α) phase at higher temperatures, i.e., above the main phase transition temperature (T_m), and an intermediate ripple phase (P_β') existing between the pre- and main transition temperatures [1–4,6–16]. The first of these is characterized by the presence of fully extended acyl chains forming a quasihexagonal lattice. The hydrocarbon chains in such assemblies are tilted through some angle with respect to the bilayer normal. In the P_β' phase, the lipid bilayer is distorted by a periodic ripple characterized by a 2D monoclinic lattice. The L_α phase has melted acyl chains [1,7,16,17]. Apart from temperature, factors such as additives, e.g., sterols, also play important roles in modifying membrane phase behavior [10,12,18–20]. These compounds optimize membranes for proper biological functioning via their interactions with phospholipids [21]. It has been well established that in the presence of low to moderate concentrations of sterols like cholesterol and phytosterols, a PC membrane possesses coexisting phases that encompass the sterol-rich and sterol-poor domains [4,12,13,15,16,19,22–24]. These domains can have different fluid characteristics and behave differently towards temperature changes.

Two important phases that are triggered by sterols are the modulated ripple phase (P_β) and the liquid ordered phase (L_o). The P_β phase is similar to the P_β' phase except in its ripple periodicity, symmetry and tilt angle [7]. A number of authors have reported the presence of such modulated phases in DMPC and DPPC membranes containing cholesterol, stigmasterol or β-sitosterol [2,4,16,25,26].

Abbreviations: DPPC, 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine or simply dipalmitoylphosphatidylcholine; DMPC, 1,2-Dimyristoyl-sn-glycero-3-phosphocholine or dimyristoylphosphatidylcholine; DSPC, 1,2-Distearoyl-sn-glycero-3-phosphocholine or distearoylphosphatidylcholine; PSO_4, sodium β-sitosteryl sulfate; Chol-SO_4, cholesterol sulfate; DSC, differential scanning calorimetry; SWAXS, small and wide angle X-ray scattering; POM, polarized optical microscopy; FF-TEM, freeze- fracture transmission electron microscopy; d, bilayer repeat distance; T_m, main phase transition maximum; T_p, pre-transition peak maximum.

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https://doi.org/10.1016/j.colsurfb.2017.10.014
0927-7765/

The L_o phase is considered to be an intermediate phase between the gel and L_α phases, and hence resembles the gel and L_α phases in chain conformation and lateral diffusion, respectively [4,10,22,27]. Sterols can induce the L_o phase either by fluidizing the gel phase or rigidifying the liquid crystalline phase [10,22]. This optimizing property of sterols is of high importance in biological functioning. It also finds applications in industrial processes, e.g., in preparing cosmetic and pharmaceutical formulations [28]. Despite the presence of numerous reports describing each kind of sterol-induced phase, a universally accepted opinion regarding the phase boundaries and exact composition of the heterogeneous domains at a particular sterol content has yet to be established [4,13,27].

Sterol sulfates are important derivatives of sterols. Some of them have been reported to be present in living cells. They are also found to possess anti-microbial properties [12,29,30]. Despite the existence of several sterol sulfates, cholesterol sulfate (Chol-SO_4) is the only species in this group that has been studied to some extent [11,12,19,30,31]. There is a wide consensus on the fundamental characteristics of sterol sulfates, namely, their position in the bilayer and their hydration properties. The bulky sulfate moiety in Chol-SO_4 occupies a position closer to the hydrophilic layer compared to cholesterol [12,30]. This sterol derivative was found to cause a greater decrease in T_m (the main phase transition temperature) of the sterol-poor component compared to that caused by cholesterol. It also causes a strong decrease in T_m of the sterol-rich component in a DPPC-CholSO_4 mixture in contrast to cholesterol, which slightly increases this temperature [11,12,19]. In addition, there is more disordering of the hydrocarbon chains because of the bulky, hydrated sulfate group. At present, there are no reports that specifically discuss the behavior of sodium β-sitosteryl sulfate (PSO_4). β-Sitosteryl sulfate (PSO_4) is similar to Chol-SO_4 in structure, except that it has an additional ethyl group in its aliphatic chain. This additional alkyl group can be expected to introduce differences in the way these two sterols are packed in the bilayers and affect the phase behaviors of PCs [19].

Based on their behavior towards PCs, sterol sulfates may provide new tools for understanding the molecular mechanisms involved in sterol-phospholipid interactions [11]. Additionally, due to their increased ability to cause disorder in PC hydrocarbon chains relative to the parent sterols, they have high prospects for being used in cosmetic and pharmaceutical formulations in combination with saturated-chain phosphatidylcholines, which are more stable than the natural, unsaturated PCs. Their capacity to enhance membrane hydration can also be of high use in industrial products. Phytosterol sulfates bear the added advantage of being derived from plant sources and hence, are safer towards the health and environmental threats such as BSE (bovine spongiform encephalopathy) infections spreadable by contaminated cholesterol and its derivatives [32]. In fact, β-sitosteryl sulfate is already in use in cosmeceutical formulations as a keratinocyte differentiation regulator [33,34]. However, details of its interactions with phosphatidylcholines have not been studied yet. Therefore, it is important to investigate different characteristics of PSO_4. In this paper, we discuss the phase behavior of DPPC in the presence of sodium β-sitosteryl sulfate.

2. Materials and methods

2.1. Materials

DPPC of 99% purity was supplied by NOF Corporation, Japan and was used without further purification. Crude samples of sodium β-sitosteryl sulfate (PSO_4) were supplied by LVMC Inc., Tokyo, Japan. Purification of PSO_4 was performed as follows.

The crude sample was first dissolved in a 1:1 mixture of hexane and acetone by heating to 50 °C. The resulting material was filtered and dissolved in ethyl acetate, again at 50 °C, with occasional sonication. The corresponding compound in ethyl acetate was filtered and dried via rotary evaporation at 70 °C for 30 min. The dried sample after removal of ethyl acetate was dissolved in methanol by heating to 50 °C. Finally, the sample was filtered and pure sodium β-sitosteryl sulfate was obtained from the filtrate by removing methanol via rotary evaporation at 70 °C and was characterized by ^{1}H NMR spectroscopy. The purity of PSO_4 freed from non-sulfated impurities was estimated to be 95–98% by HPLC.

2.2. Sample preparation

Appropriate quantities of DPPC and sodium β-sitosteryl sulfate were weighed to obtain mixtures of varying compositions such that the mole fraction of PSO_4 (x) varied from 0 to 0.5. The mixtures were then dissolved in a solvent containing chloroform and methanol in a volume ratio of 3:1. The solvents were removed by passing a stream of nitrogen gas through the solution. The samples were then vacuum-dried. Water was added to obtain mixtures with 40 wt% of lipids. The mixtures were then subjected to three cycles of annealing followed by homogenization by heating at ~60 °C (about 20 °C above T_m) with vortexing and stirring. For ensuring proper homogenization, the samples were also stirred with a spatula after cooling to room temperature. The test tubes containing these samples were then sealed tightly and incubated. Measurements were completed with the equilibrated samples within 2–3 weeks from sample preparation.

2.3. Polarized optical microscopy (POM)

Small amounts of samples were placed on clean and dry glass slides and cover slips were carefully lowered on the samples, in an inclined position, with the help of a narrow spatula to avoid any entrapping of air bubbles. They were then gently pressed to achieve appropriate thickness. The samples were observed using an IMT-2 microscope (Olympus Optical Co. Ltd.) [35] equipped with a temperature control unit (Mats-1002RO, Tokai Hit Co., Ltd.), through crossed polarizers. The resulting birefringent textures were transferred to a computer with the help of a Moticam 2000 digital camera fitted on the eyepiece. The textures were used to characterize lamellar phases as well as to see the changes brought about due to the addition of PSO_4.

2.4. Differential scanning calorimetry (DSC)

DSC measurements were carried out on a Rigaku DSC-8230 instrument. Measurements were performed during a continuous temperature scan from 10 °C to 85 °C. About 3.0-3.3 mg of samples were sealed in aluminum sample pans and the heat flow was measured against alumina as a reference. Two heating and cooling cycles were performed for each sample at rates of 3 and 1 $Kmin^{-1}$. The 1 $Kmin^{-1}$ run was used for studying the general transition behavior, whereas the 3 $Kmin^{-1}$ run was used for studying the variation of pre-transition, which is a low-enthalpy transition (particularly in the presence of PSO_4), and hence, is likely to be missed at a slow scan rate [4].

2.5. Small and wide angle X-ray scattering (SWAXS)

Scattering experiments were carried out using a W 3830 X-ray generator (PANalytical Co., Ltd., Almedo, Netherlands) and diffraction patterns were recorded on a SAXSess camera (Anton Paar Co., Ltd., Graz, Australia) in a line collimation system. A semitransparent beam stop was available to attenuate the beam. The samples were placed in a vacuum-proof metallic cell between Mylar windows and the cell was tightened at both ends. Each sample was exposed