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Colloids and Surfaces A: Physicochem. Eng. Aspects 293 (2007) 123–129
COLLOIDS AND SURFACES A
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Condensation effect of cholesterol, stigmasterol, and sitosterol on dipalmitoylphosphatidylcholine in molecular monolayers

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Received 2 November 2005; received in revised form 5 July 2006; accepted 11 July 2006
Available online 14 July 2006

Abstract

The effects of the incorporation of cholesterol, sitosterol, and stigmasterol into dipalmitoylphosphatidylcholine (DPPC) monolayers were investigated by Langmuir monolayer film technique in this study. The compressibility coefficient was assessed at various surface pressures. The observations indicated that sitosterol and stigmasterol interacted less effectively than cholesterol with the phospholipid. Nevertheless, these sterol molecules could all cause condensation effect on DPPC monolayers. Attractive interactions between DPPC and sterol molecules, or hydrophobic effect, was found to play an important role, testified by negative excess molecular areas at particularly low surface pressures and negative partial molecular area of three sterols at low surface pressures. The minimum extreme points for the excess area were all located at around 0.3 mol fractions for the three sterols at 30 mN/m, which suggest a 2:1 ratio of DPPC/sterol in the ordered structures.

Keywords: Condensation effect; Cholesterol; Stigmasterol; Sitosterol; Monolayer

1. Introduction

Sterols play crucial regulatory and structural roles in the lateral organization of eukaryotic cell membranes. Among them, cholesterol has been connected to the formation of ordered lipid domains or rafts in mammalian cell membranes [1]. It is now well accepted that lipid rafts are composed of lipids in the liquid-ordered (L_o) phase and are surrounded with lipids in the liquid-disordered (L_d) phase [2]. This state is characterized by tight packing, similar to that in the more solid-like gel state, but also fast lateral motion, although slightly slower than that observed in the more disordered liquid-crystalline state. The tight packing of lipids in the L_o state gives rafts their characteristic resistance to cold, nonionic detergents such as Triton X-100. Cholesterol and sphingomyelin (SM) are thought to be the principal components of lipid rafts in cell and model membranes. These rafts are believed to be involved in biological processes such as membrane fusion, signal transduction, and virus release at the cell membrane level [3–10].

Among saturated phosphatidylcholines, dipalmitoylphosphatidylcholine (DPPC) has been an especially model raft lipid because its saturated acyl chains and phosphorylcholine head group duplicate some of the important structural features of SM, and DPPC does form L_o phase upon mixing with adequate amounts of cholesterol [11,12]. Examination of the thermal properties of mixed aqueous dispersion of cholesterol with DPPC showed that increasing proportions of cholesterol caused a progressive broadening of the gel to liquid-crystalline phase transition and a decrease in enthalpy change of the transition [13].

Most studies on sphingolipid/sterol mixtures aim an understanding of its role in mammalian cells. However, detergent resistant sphingolipid/sterol membrane fractions have also been isolated from higher plants such as tobacco leaves [14,15] and Arabidopsis root-derived callus [16]. Tobacco lipid rafts were found to be greatly enriched in sphingolipid, as well as in sterol mixtures including stigmasterol and sitosterol. Plant sterols, or phytosterols, have been reported to include over 250 different sterols and related compounds in various plant and marine materials. Sitosterol and stigmasterol are two of the most common representatives. Compared to cholesterol, stigmasterol and sitosterol (Fig. 1) have an ethyl group at carbon 24. Stigmasterol

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doi:10.1016/j.colsurfa.2006.07.016

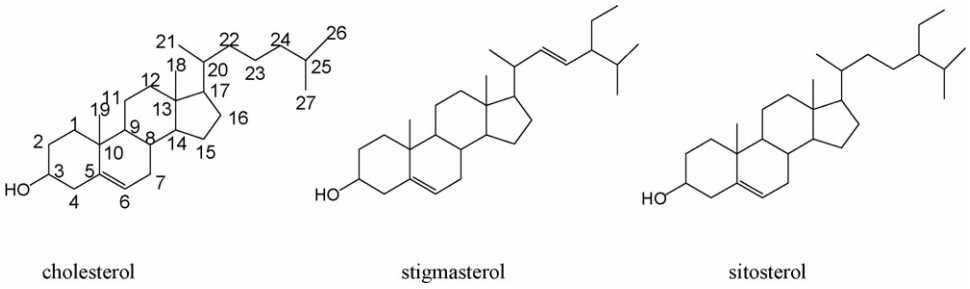

Fig. 1. Molecular structures of cholesterol, stigmasterol, and sitosterol.

furthermore contains an additional *trans* double bond between carbons 22 and 23. The aim of this work is to answer questions such as if the phytosterols can play identical role as cholesterol in forming ordered structures with DPPC and what stoichiometry between the phytosterols and DPPC is, knowing that cholesterol and DPPC can form L_o phase with 1:2 molecular ratio [17–20]. The work also aims to clarify the molecular mechanism of the condensing effect of sterols, particularly to differentiate possible void-filling effect and hydrophobic effect between the sterols and DPPC.

2. Experimental

2.1. Materials

DPPC (purity >99%) was purchased from Avanti Polar-Lipids, Inc. (Birmingham, AL). Stigmasterol and sitosterol were bought from MP Biomedicals, Inc. (Germany). Cholesterol was purchased from Beijing Chemical Reagent Co. (China). The samples were used without further purification. *n*-Hexane and isopropanol from Beijing Chemical Industrial Co. (China) were of analytical grade and used as received.

2.2. Monolayer formation

A commercially available computer-controlled KSV mini-trough (KSV Instruments Ltd., Finland) equipped with a platinum Wilhelmy plate was used to acquire the surface pressure–molecular area (π–A) isotherms of monolayers at $25 \pm 1\,^{\circ}C$. The water used as subphase was purified by Shuang Feng purification system (Beijing, China) to yield a product with a resistivity of 18.2 MΩ cm. The purity of the subphase was checked prior to lipid spreading by compressing the surface down to 5% of its initial area. The surface pressure was measured during the compression, and the solution was considered clean if the rise in π did not exceed 0.2 mN/m. The subphase was maintained at fixed temperature using a thermostatic, circulating water bath.

DPPC and sterols were both dissolved in *n*-hexane/isopropanol (7/3, v/v) mixed solvents. They can dissolve the lipids well and is volatile and less toxic. The concentration of the samples was controlled to around 0.5 mg/ml. DPPC was mixed with sterol in different molar ratios and spread onto the surface of the subphase using a micrometric syringe. The spreading volume was 40 μl. 15 min was allowed for the evaporation of solvent before the π–A isotherms were recorded with a compression rate of 10 mm min^{-1} (750 mm^2 min^{-1}).

2.3. Analysis of isotherms

Two-dimensional compressibility of the monolayer has been investigated. The following Eq. (1) was used for the calculation of the compressibility coefficient [21].

$$\beta = -\frac{1}{A}\left(\frac{\partial A}{\partial \pi}\right)_T \tag{1}$$

Four or five parallel isotherms were run to get reliable compressibility coefficient. It is worthwhile to point out that the quantity expressed in the above equation is actually the reciprocal value of compressional moduli (Cs^{-1}).

The ideal value of the molecular area for the mixed DPPC/sterol monolayer, A_m^i, can be calculated from the molar ratio of the two components $A_m^i(x) = xA_{\text{sterol}}^* + (1-x)A_{\text{DPPC}}^*$, where A_{sterol}^* and A_{DPPC}^* are the molecular areas of pure sterol and DPPC, respectively, at a given surface pressure. A smoothing equation according to Zegers and Somsen's local fitting procedure [22] is chosen for fitting the experimental results of molecular area against sterol mole fraction x at each surface pressure: $A_m(x) = b_1 + b_2x + b_3x^2$. b_1, b_2, and b_3 are constants and b_1 is the molecular area of DPPC. The excess molecular area, A_m^E, which is the difference in molecular area between the fitted value and the ideal value, is given by:

$$A_m^E(x) = (b_2 - A_{\text{sterol}}^* + A_{\text{DPPC}}^*)x + b_3x^2 \tag{2}$$

The partial molecular areas $A_{\text{sterol},m}$ and $A_{\text{DPPC},m}$ of the components are calculated by using equations [23]:

$$A_{\text{sterol},m} = A_{\text{sterol}}^* + A_m^E + (1-x)\left(\frac{\partial A_m^E(x)}{\partial x}\right)_{T,\pi} \tag{3}$$

$$A_{\text{DPPC},m} = A_{\text{DPPC}}^* + A_m^E - x\left(\frac{\partial A_m^E(x)}{\partial x}\right)_{T,\pi} \tag{4}$$

Further, excess partial molecular areas A_i^E of the component i can be evaluated by subtracting the pure molecular area of the component i from its corresponding partial molecular area using equation: $A_{i,m}^E = A_{i,m} - A_i^*$.