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A concentration dependent spectroscopic study of binary mixtures of plant sterol stigmasterol and zwitterionic dimyristoyl phosphatidylcholine multilamellar vesicles: An FTIR study

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ABSTRACT

Plant sterols such as stigmasterol and sitosterol are mostly found in plant cell walls and membranes and have been used as cholesterol-lowering agents as well as anti-inflammatory, antibacterial, antifungal, anti-ulcerative and antitumor activities. This study was conducted to examine the thermotropic and physicochemical properties of binary mixtures of plant sterol stigmasterol and zwitterionic dimyristoyl phosphatidylcholine (DMPC) multilamellar vesicles (MLVs) as a function of temperature and different stigmasterol concentrations by utilizing Fourier transform infrared (FTIR) spectroscopy. In this study, we observed that stigmasterol incorporation induces variations in the lipid thermotropic phase behavior, lipid order (acyl chain flexibility) and dynamics (fluidity) and hydration state of the head group and/or the region near the head group of DMPC MLVs. Our results suggest that stigmasterol is fully capable of interacting with zwitterionic DMPC membranes and changes the biophysical properties of them.

1. Introduction

Plant sterols known as phytosterols are integral natural ingredients of plant cell membranes and are structurally and functionally similar to cholesterol in vertebrates [1,2]. The most well-known plant sterols are β-sitosterol, stigmasterol, and campesterol, which are classified as 4-desmethylsterols of the cholestane series and they have double bonds at the C5 position of ring structure as cholesterol, but with one or two additional carbon atoms in the alkyl side chain [3–5]. While campesterol has a methyl group at C24 of the alkyl side chain, β-sitosterol has an ethyl group at that position. Stigmasterol is analogous to β-sitosterol, but it has an additional double bond between C22 and C23 [5]. The chemical structures of β-sitosterol, stigmasterol, and campesterol are shown in Fig. 1.

Plant sterols can be found in all food items of plant origin. It is known that they have many bio-active properties for the health of humans. Its the serum cholesterol lowering effect, which is the best known effect among the other known properties, inhibits the intestinal absorption of cholesterol which results in lowering total plasma cholesterol and LDL (low-density lipoprotein) levels [1,5]. Plant sterols also might have an inhibitory effect for development of colon cancer. The main plant sterol, β-sitosterol, has been proved that it inhibits human cancer cell growth, has a dose-dependent effect on the epithelial cell proliferation, and also causes a decrease in the number of tumours developed in the colon [5]. In addition to the mentioned effects, plant sterols have shown to possess antibacterial [6], antifungal [7], anti-ulcerative [8], anti-atherosclerotic [9], anti-inflammatory [10] and anti-oxidative activities [11]. Therefore, the effects of phytosterols on human health play essential roles in the treatment and prevention of several diseases and the studies related to phytosterols at clinical level or in model systems are very important to understand the molecular mechanism behind such diverse function of these compounds.

A number of studies are available in the literature about the interaction of plant sterols with membrane components and specifically with lipids by using several biophysical and structural techniques [12–17]. These studies explore the effects of plant sterols in phospholipid membranes, such as effects on the thermotropic properties of phospholipids [12,13,16,17] and on the phospholipid order and condensation [14,15]. However, the

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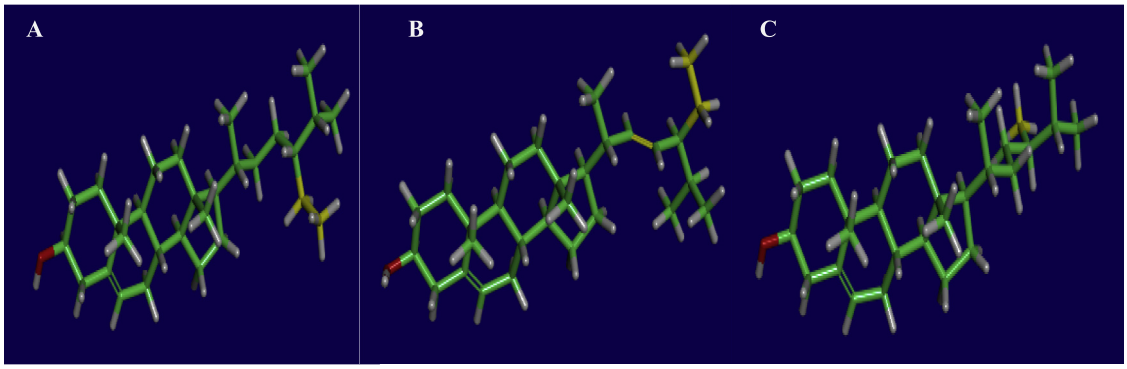

Fig. 1. Chemical structures of β-sitosterol (A), stigmasterol (B) and campesterol (C). The hydroxyl group at carbon 3 is red, and the ethyl group at carbon 24 for β-sitosterol, the *trans* double bond at carbon 22 and the ethyl group at carbon 24 for stigmasterol, and the methyl group at carbon 24 for campesterol are yellow. These structures were generated with the Discovery Studio 4.5 Client (Accelrys, Inc., San Diego, CA, USA). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

findings about the binary mixtures of plant sterol stigmasterol and zwitterionic DMPC membranes are still missing. Due to the lack of these findings, for the first time, we applied a noninvasive technique, Fourier transform infrared (FTIR) spectroscopy, in order to understand the concentration dependent effects of stigmasterol on DMPC membranes. The findings of our experimental study allowed us to determine the structural and dynamical changes such as lipid phase behavior, order and dynamics, and nature of hydrogen bonding after incorporating stigmasterol into DMPC membranes as a function of temperature. Our findings showed that plant sterol stigmasterol has some different effects on DMPC membranes depending on concentration.

2. Materials and methods

2.1. Chemicals

Stigmasterol, DMPC and phosphate buffered saline (PBS) tablets were purchased from Sigma (St. Louis, MO, USA). All chemicals were obtained from commercial sources at the highest grade of purity available.

2.2. FTIR studies

For the infrared measurements, pure phospholipid MLVs were prepared according to the procedure reported by Severcan et al. [18]; 5 mg of phospholipid was dissolved in chloroform in a round-bottomed flask. A dried lipid film was obtained by evaporating with a nitrogen flux and then pumping for at least 2 h under vacuum with a CHRIST RVC 2−18 CD spin vac (CHRIST, Osterode am Harz, Germany). The film was hydrated by adding 25 μl of PBS buffer solution, pH 7.4. Liposomes were formed by vortexing the mixture at a temperature above the gel-to-fluid phase transition for 20 min. To prepare stigmasterol containing liposomes, the appropriate amount of stigmasterol was taken from a stock solution, in which stigmasterol was dissolved in chloroform and put in a round-bottomed flask. Excess chloroform was evaporated by nitrogen stream and then 5 mg of DMPC was added and dissolved in chloroform in the same round-bottomed flask. The same procedure for the preparation of pure DMPC liposomes was then followed. The PBS solution used that we used during the experiments was obtained by dissolving the PBS tablets in deionized water, resulting in a solution of 0.01 M phosphate buffer with 0.0027 M potassium chloride and 0.137 M sodium chloride with a pH of 7.4 [19–21]. For FTIR studies, sample suspensions of 20 μl were placed between CaF_2 windows with the cell thickness of 12 μm. Infrared spectra were obtained using a PerkinElmer Frontier FTIR spectrometer (Perkin−Elmer Inc., Norwalk, CT, USA) equipped with a deuterated triglycine sulfate (DTGS) detector. Interferograms were averaged for 50 scans at 2 cm^{-1} resolution. Temperature was regulated by a Graseby Specac (Kent, UK) digital temperature controller. The samples were incubated for 10 min at each temperature before data acquisition. Samples were scanned between 0 and 40 °C with 2 °C intervals. The spectra were analyzed using PerkinElmer Spectrum v10.3.7 software (Perkin−Elmer Inc., Norwalk, CT, USA). The spectrum of the air was recorded as a background spectrum and subtracted automatically from the spectra of samples by using the PerkinElmer Spectrum v10.3.7 software, which was also used for data analyses. Since the OH stretching bands due to the buffer appear in the regions of 3400−3200 cm^{-1} and 1800-1500 cm^{-1}, these bands overlap with the bands of interest. Therefore, the spectrum of the buffer was taken at different temperatures and was subtracted from the spectrum of liposomes at corresponding temperatures. The subtraction process was performed until the bulk water region located around 2100 cm^{-1} was flattened using the PerkinElmer Spectrum v10.3.7 software program. The band positions were measured in accordance with the center of weight and bandwidth was measured at 0.80 × peak height position. The detailed analyses were performed from the subtracted native spectra. However, for visual demonstration of the spectral differences in the spectra, the spectra were normalized with respect to the specific bands.

3. Results and discussion

In this study, the influence of stigmasterol on the structure and dynamics of DMPC MLVs was detected as a function of temperature and different stigmasterol concentrations by FTIR spectroscopy by monitoring various functional groups, such as C−H stretching at 2800-3000 cm^{-1}, C=O stretching at 1735 cm^{-1} and PO_2^- antisymmetric double stretching bands at 1220-1240 cm^{-1}. The spectral variations with regard to these vibrational modes illustrated in Fig. 2A become characteristic data, because these ensure precious structural and conformational knowledge about the thermotropic variations which take place in the hydrocarbon chains and the interfacial and the head group regions of DMPC, respectively [18,22,23]. Increasing concentrations of stigmasterol were favored as 1, 5 and 10 mol% (low concentrations) and 20, 30 and 40 mol% (high concentrations) to investigate the concentration-dependent behavior of stigmasterol on DMPC MLVs. The normalized FTIR spectra of DMPC MLVs in the presence and absence of stigmasterol at low (10 mol%) and high (40 mol%) concentrations in the liquid

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