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The effect of sterol derivatives on properties of soybean and egg yolk lecithin liposomes: Stability, structure and membrane characteristics

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ABSTRACT

The effects of three kinds of sterols (cholesterol, β-sitosterol and ergosterol) on the stability, microstructure and membrane properties of soybean and egg yolk lecithin liposomes were investigated by light scattering, transmission electron microscope (TEM), atomic force microscope (AFM), fluorescence and Fourier transform infrared spectroscopy (FTIR). The vesicle size of cholesterol or β-sitosterol incorporated liposomes was higher than that of the control and ergosterol incorporated ones, while the zeta-potential was similar when the same lecithin was used. Due to the excellent emulsifying capacity, Tween-80 was introduced into the system and which could obviously maintain the liposomal vesicle size in fetal bovine serum. According to TEM and AFM, the phenomena of membrane fusion and deformation were observed respectively in ergosterol-incorporated liposomes. Results of fluorescence probe spectra revealed the most compact membrane structure was found in cholesterol-incorporated liposomes, which was in accordance with the strongest intermolecular interaction in bilayers obtained by FTIR results. Conversely, the membrane of ergosterol-incorporated liposomes was the most fragile and fluid, which was also identified with the lowest physical stability obtained by Turbiscan. These results systematically illustrated the relationship between the structure of sterols and the liposomal membrane stability, and provided some meaningful information on the choice of sterols and lecithin in preparation of liposomes for different purposes.

1. Introduction

As a potential nutrient delivery system, spherical-shaped liposomes, which contain internal aqueous phase surrounded by one or more concentric phospholipid bilayers, have been applied to encapsulate hydrophilic, lipophilic and amphiphilic bioactive compounds simultaneously. Compared with traditional emulsion delivery system, liposomes own numerous advantages such as biodegradability, biocompatibility, nontoxicity and non-immunogenicity (McClements, 2015). Nevertheless, the irreversible degradation of phospholipid and thermodynamic instability severely limit the application of liposomes in pharmacy, cosmetic and food industries. Therefore, the liposomal stability has received much attention in recent studies. Most of these researches focused on the effect of coating layers on the surface of liposomal vesicles, such as polysaccharides (Toniazzo et al., 2014; Zhou et al., 2014), polyethylene glycol (Abe et al., 2015) and protein (Frenzel & Steffen-Heins, 2015). However, these coating protocols could increase the material cost and complexity of preparation processing.

Sterols are well known as the essential lipophilic component in formation of liquid-ordered membrane state, which could play an essential role in regulating the membrane characteristics like protein translocation. In consideration of the diverse molecular structures of sterols, the self-assembling mechanism in different sterol-phospholipid membranes is necessary to be clear. The cholesterol derived from zooblast has been widely used in artificial membranes for increasing the mechanical strength and reducing the permeability. In recent years, phytosterol (such as β-sitosterol), which is identified to be beneficial for health, has been considered as a potential alternative compound for cholesterol due to the production capacity of lipophilic heart-healthy compounds (Alexander, Lopez, Fang, & Corredig, 2012). Except for phytosterol, the emerging fungisterol (such as ergosterol) has also been applied in the study of liposomal preparation (Bui, Suga, & Umakoshi, 2016; Chen & Tripp, 2012). The fundamental molecular structures of these typical sterol derivatives above are similar apart from several carbon sites of benzene ring and alkyl side chain. When sterol was incorporated into phospholipid bilayers, hydrophilic groups attached on the benzene ring preferred to the aqueous phase, while the alkyl chain tended to the deep hydrophobic area (Chen & Tripp, 2012). Bui et al.

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(2016) comparatively studied the effect of three kinds of sterols on the fundamental properties of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) membrane, they found that cholesterol was more effective than lanosterol and ergosterol in ordering the hydrophobic region of membrane. Chen and Tripp (2012) also found that the molecular structure of sterol dominantly influenced the conformational order of liposomal membrane. With respect to the delivery of bioactive compounds in liposomes, insulin (Cui et al., 2015), natamycin (Bouaoud, Lebouille, Mendes, De Braal & Meesters, 2016), oligopeptides (Hwang, Tsai, & Hsu, 2010) and ascorbic acid (Alexander et al., 2012) have been used as model molecules to investigate the encapsulation efficiency or release property of sterol-phospholipid membrane. These correlational studies revealed that sterol derivatives played an important role in liposomal constructions no matter what kind of bioactive compound was encapsulated. Given the above, few studies focused on the relationship among stability, morphology, membrane property of liposomes containing different sterols.

The main purpose of this study was to establish the relationship between characteristics of sterol-phospholipid bilayers and stability of vesicles. In this framework, soybean and egg yolk lecithin liposomes containing cholesterol, β-sitosterol and ergosterol were prepared by the thin film hydration-sonication method. A comprehensive vesicle characterization, physical stability, morphology, membrane property and intermolecular interaction were determined by light scattering, transmission electron microscope (TEM), atomic force microscope (AFM), fluorescence spectroscopy of three kinds of probes (Pyrene, ANS and DPH) and Fourier transform infrared spectroscopy (FTIR) respectively. The results could provide theoretical basis on the formation mechanism of liposomal vesicles for different purposes of encapsulation and release.

2. Materials and methods

2.1. Materials

Phospholipon® 90G (soybean lecithin at 96.7% of phosphatidylcholine, 1% of lysophosphatidylcholine, 0.3% of water and 0.1% of ethanol) and Lipoid E80 (egg yolk lecithin at 82.8% of phosphatidylcholine, 7.9% of phosphatidylethanolamine, 2% of lysophosphatidylcholine, 2.5% of sphingomyelin, 2.5% of triglycerides, 1% of phosphatidylinositol and 0.5% of water) were purchased from Lipoid GmbH (Ludwigshafen, Germany). Cholesterol was obtained from Sinopharm Chemical Reagent Co., Ltd. (China). β-sitosterol ($>$ 75%) and ergosterol ($>$ 95%) were purchased from Aladdin biological technology Co., Ltd. (Shanghai, China). Pyrene ($\geq$99%), 8-Anilino-1-naphthalenesulfonic acid (ANS, $\geq$95%) and 1,6-Diphenyl-1,3,5-hexatriene (DPH, 98%) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) was obtained from Biological Industries Israel BeitHaemek Ltd. Tween-80 was purchased from Xi Long Chemical Co., Ltd. (China). All other chemicals used were analytical grade, unless otherwise stated.

2.2. Preparation of liposomes

Different liposomes were prepared using the thin film hydration-sonication method according to our previous study (Tai et al., 2017). Briefly, 10 mg/mL of lecithin was mixed with sterol at a mass ratio of 5:1 in chloroform/methanol (2:1, v/v) solution and the mixture was vacuum-desiccated to form thin lipid film using rotary evaporator at 50 °C. The dried film remained under vacuum for 30 min to remove residual organic solvent. Since then, it was rehydrated with phosphate buffer solution (PBS, 0.01 M, pH 7.4) containing Tween-80 (1:50, w/v) or not at temperature above the main phase transition of lecithin. To reduce the vesicle size, the coarse liposomal suspension was subjected to sonication using probe-type sonicator at 400 W for 2 min. The prepared liposomes were stored in refrigerator (4 °C). Samples of the liposomes prepared using soybean lecithin, soybean lecithin-cholesterol, soybean lecithin-β-sitosterol and soybean lecithin-ergosterol were termed as SL, SL-Chol, SL-Sito, SL-Ergo, respectively; Samples of the liposomes prepared using egg yolk lecithin, egg yolk lecithin -cholesterol, egg yolk lecithin-β-sitosterol and egg yolk lecithin -ergosterol were termed as EL, EL-Chol, EL-Sito, EL-Ergo, respectively.

2.3. The vesicle size and zeta potential measurements of liposomes

The vesicle size was determined by dynamic light scattering (DLS) using Malvern ZetasizerNano-ZS90 (Malvern Instruments Ltd., Worcestershire, UK), equipped with He/Ne laser operating at a wavelength of 633 nm, the scattering angle was 90°, the refractive index was 1.490. *Z*-average diameter was recorded as the vesicle size which means the hydrodynamic size of liposomes.

Zeta-potential (ζ) was determined according to the Smoluchowski relation of the ionic mobility with the surface charge. Before measurement, all samples were diluted 10-fold in phosphate buffer solution (0.01 M, pH 7.4) to avoid multiple scattering. The pH during measurements was kept consistent with the hydration medium used in the preparation of liposomes to avoid the occurrence of pH differences between the internal aqueous phase and the external medium in liposomes, which could break the basic liposomal structure. Each measurement was carried out in triplicate at 25 °C after 2 min of equilibration.

2.4. Liposome morphology

2.4.1. Transmission electron microscopy (TEM)

According to the method described by Liu et al. (2015), the liposomal vesicle morphology was observed using JEM-1200EX TEM (Japanese Electronics Co., Ltd., Japan) at an accelerating voltage of 100 kV. One drop of diluted liposomes suspension was placed onto a 200-mesh carbon coated copper grid. After that, negative staining was conducted using uranyl acetate solution (3%) for 90 s and air-dried at 25 °C. The excess liposomes suspension and uranyl acetate solution were removed by filter paper if necessary.

2.4.2. Atomic force microscopy (AFM)

AFM images could illustrate the surface morphology and elastic properties of liposomal vesicles (Zhao et al., 2015). The liposomes were diluted 500-fold in PBS (0.01 M, pH 7.4) and a drop of diluted sample was deposited onto a cleaved mica slide and air-dried at room temperature for $>$ 3 h before determination. Imaging was conducted in tapping-mode at scan rate of 0.8 Hz using a NTEGRA Solaris AEF (NT-MDT, Russia) and RTESPA tapping tips (Bruker). The resonance frequency was set at 300 kHz and the force constant was 40 N/m.

2.5. Stability assay

2.5.1. The stability of liposomes in fetal bovine serum solution (FBS)

When liposomes were absorbed into the blood, surface profile and particle size of liposomal vesicles could be easily influenced by serum. In order to confirm the effect of Tween-80 and sterol on the stability of liposomes in serum solution, all freshly prepared liposomes were incubated in FBS-PBS solution with different concentrations (1%, 2.5%, 5% and 10%, pH 7.4) for 4 h. The variation of vesicle size was measured by DLS as described above without dilution.

2.5.2. The physical stability

Due to the disassembling and fusion of the membrane, vesicles could be precipitated in liposomes easily. The physical stability was evaluated by multiple light scattering using Turbiscan Lab Expert (Formulaction, L'Union, France) equipped with a pulsed near-infrared light source (λ = 880 nm). The light pulses transmitted and backscattered signals (at 180° and 45° from the incident beam respectively)

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