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Charge boosting effect of cholesterol on cationic liposomes

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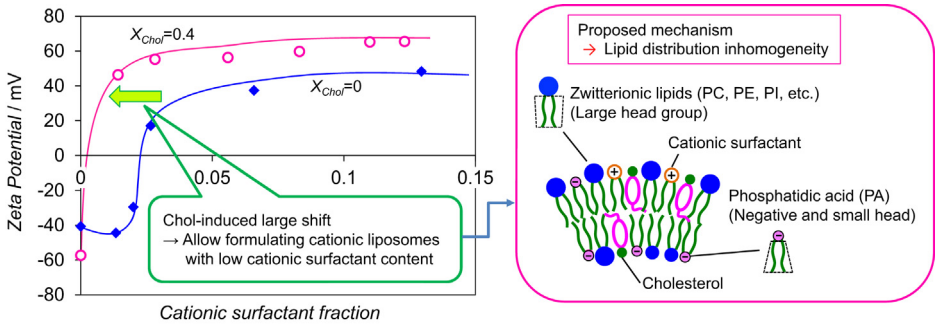
HIGHLIGHTS

- Effect of cationic molecular structures on the liposomes zeta potential was studied.
- Cholesterol mixing enhanced the positive zeta potential of cationic liposomes.
- Cationic liposomes can be formulated with less cationic surfactant content.
- Chol-induced phase transition in liposome membrane is responsible.
- A new strategy to construct cationic liposomes with low cytotoxicity was proposed.

GRAPHICAL ABSTRACT



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ABSTRACT

Cationic liposomes that have been used as drug or gene delivery vehicles can be obtained by mixing cationic surfactants in phospholipid liposomes. Cytotoxicity of cationic molecules is one of the problems of the cationic nanocarriers. Therefore we aimed to formulate highly positively-charged cationic liposomes with low content of cationic surfactant, which have both higher cellular uptake ability and lower cytotoxicity. We have investigated the zeta potential behavior in water/soy bean lecithin/cationic surfactant/cholesterol systems by the changing composition as well as the molecular structure of the cationic surfactant. Mixing of monoalkyl (C_{12}, C_{16} and C_{18}) and dialkyl (C_{12} and C_{18}) quaternary ammonium chlorides were increased the zeta potential of liposomes from negative to positive values (more than +50 mV). Mixing the cholesterol into the cationic liposomes increased the maximum zeta potential further and also induced the large shift of the negative-positive transition point with respect to the cationic surfactant mixing fraction. It means much less cationic surfactant is needed to obtain highly positively-charged liposomes. The significant cholesterol effects on the zeta potential of liposomes were explained based on the lipid distribution inhomogeneity caused by the increased fluidity of liquid ordered phase membranes and the large critical packing parameter of the negatively-charged lipid.

1. Introduction

Cationic liposomes or vesicles show enhanced drug delivery efficiency [1–6] as well as the ability to deliver negatively charged biomolecules such as nucleic acids, proteins and peptides [7–14] since the positive surface charge is suitable for cellular uptake [15–17]. Cationic liposomes can be obtained by mixing cationic

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surfactants such as alkyl quaternary ammonium salts [18,19] or cationic lipids such as cationic cholesterol derivatives [20–24] with phospholipid liposomes that have negative or weakly positive zeta potential. In addition, cholesterol is generally mixed in liposomes. Cholesterol is an important component of the cell membrane. Cholesterol changes cell membrane fluidity and contributes to stabilization of the cell membrane and regulation of material transport through the cell membrane. Mixing cholesterol in phospholipid membranes induces the phase transition to the liquid ordered (L_O) phase, which is an intermediate phase between the liquid disordered (liquid crystalline) phase and the crystalline phase [25]. L_O phase formation is not limited to phospholipid systems but is also observed in lysophospholipid and general surfactant systems [26–28]. Even if cholesterol is a nonionic molecule, it may affect the dissociation of cationic amphiphiles in the cationic liposome membrane through changing the conformational disorderliness of the cationic head group area, which is suggested by molecular dynamics simulation [29]. Actually, increasing zeta potential of cationic liposomes for an antigen carrier is reported [30] as well as other similar examples [31].

The cationic liposomes are important for medical and biological applications since the positive surface charge enhances the delivery efficiency of drugs or biomolecules because of enhanced cellular uptake. However, cytotoxicity of cationic molecules is one of the drawbacks of cationic nanocarriers [15–17]. To solve this problem, reducing the content of cationic molecules can be a solution. Cationic molecules can contribute the positive charge of liposomes when they are incorporated in the liposome membrane. Therefore, we have to know the efficient cationic molecular structure with respect to the efficient incorporation in the liposome membrane. Also the degree of counter ion dissociation is important. In this study, firstly we investigated zeta potential behavior of liposomes formed in the water/soy bean lecithin/cationic surfactant systems with respect to the cationic surfactant composition and molecular structure. Then, we added cholesterol to the efficiently-charged cationic liposomes and found that the role of cholesterol was a key to formulate highly positively-charged cationic liposomes with low content of cationic surfactant molecules. The present complex liposomal system with lecithin (a phospholipid mixture), cationic surfactant and cholesterol is worth studying since the synergistic effect of three different amphiphilic ingredients has not been understood.

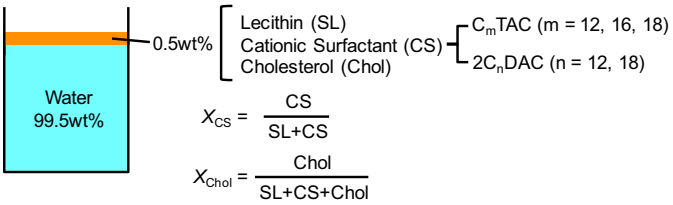
2. Materials and methods

2.1. Materials

Hydrogenated soybean lecithin (SL), which contains several different phospholipids, such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidic acid, was obtained from YMC Ingrid, Japan. Dodecyltrimethylammonium chloride (C_{12}TAC), hexadecyltrimethylammonium chloride (C_{16}TAC), octadecyltrimethylammonium chloride (C_{18}TAC), didodecyldimethylammonium chloride ($2C_{12}$DAC) and dioctadecyldimethylammonium chloride ($2C_{12}$DAC) were purchased from Tokyo Chemical Industry (TCI), Japan. Cholesterol (Chol) was also purchased from TCI. All the chemicals were used as received. Millipore filtered water was used as the solvent.

2.2. Preparation of liposomes

Liposomes were prepared by the thin-film (Bangham) method [3]. Amphiphilic molecules were weighed in eggplant flasks with sufficient chloroform to make isotropic solutions. The chloroform was removed by a rotary evaporator and solid mixtures of the lipids and surfactants were obtained as thin-film covering the bottom of the flasks. Water was added to the flask to make the final composition 99.5 wt% of water. After 2 h of water swelling in the thin film, ultrasound treatment was performed using the probe-type ultrasonicator (USP-50, Sonics & Materials, USA) at 20 kHz, 50 W for 15 min. Since the system contains 4 components, we presented the Scheme 1 to describe the composition of the system and the variables (X_{CS} and X_{Chol}) for better readability.



Scheme 1. Schematic representation of the composition of the systems studied.

2.3. Zeta potential measurement

The zeta potential was determined using the ELS-6000 (Otsuka Electronics, Japan) at 84 V of the electric field and 20° of the scattering angle. We repeated more than 3 times for each sample. The experimental error was within 2%. All the measurements were performed at 25 °C.

2.4. Dynamic light scattering (DLS)

DLS measurements were performed using DLS-7000 (Otsuka Electronics, Japan). The DLS apparatus consists of a goniometer, 75 mW Ar ion laser (λ = 488 nm), and a multiple tau digital real-time correlator (ALV-5000, EPP, Germany). The scattering angle was chosen as 90°. The experimental correlation function $g_2(\tau)$ was measured and fitted by a nonlinear fitting program to obtain the diffusion constant. Apparent stokes diameter was calculated by the Stokes-Einstein equation. We repeated more than 3 times for each sample. The experimental error was within 10%. All the measurements were performed at 25 °C.

2.5. Differential scanning calorimetry (DSC)

A heat flux-type DSC instrument (DSC6200, Seiko Instruments, Japan) was used. Samples (10–20 mg) containing 60 wt% of water were introduced into aluminum pans and then tightly sealed. DSC traces were recorded from −80 °C to 80 °C at a heating rate of 10 °C/min after keeping each sample at −80 °C for 30 min.

2.6. X-ray scattering

X-ray scattering measurements were performed on a Kratky-type camera (SAXSess, Anton Paar, Austria) with a PW3830 laboratory X-ray generator (Philips, Netherlands). It has a long fine focus sealed glass X-ray tube (Cu-Kα wavelength of 0.1542 nm). The apparatus was operated at 40 kV, 50 mA and an X-ray beam was irradiated on samples for 10 min. Samples containing 60 wt% of water were introduced in a quartz capillary (1 mm thick) specially designed for the SAXSess camera. All the measurements were performed at 25 °C.

3. Results and discussion

In this study, we confirmed the formulation of cationic liposomes in quaternary systems containing water, lecithin, cationic surfactant, cholesterol by using zeta potential measurements. The