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Polysaccharide-anchored fatty acid liposome

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ABSTRACT

In this study, the preparation of *N*-pamitoyl chitosan (ChP) anchored oleic acid (OA) liposome was demonstrated. Two different types of water-soluble ChPs with different degrees of acylation (DA) were selected for this study. The presence of ChPs on the surface of OA liposome was confirmed with their micrographs and physicochemical properties. The “peeling off” effect on the surface of the ChP-anchored OA (OAChP) liposomes was observed on the atomic force microscope micrographs and confirmed the presence of the ChPs layer on the liposome surface. The surface tension of the OAChPs liposome solution was found to be higher than that of the OA liposome solution. This result indicated the removal of OA monomer by ChPs from the air–water interface. The increase in the minimum area per headgroup (A_{min}) of the OA with the presence of ChPs has further proved the interaction between OA monomer and the hydrophobic moieties of the ChPs. The ChPs anchored onto the OA monolayer increased the curvature of the OAChP liposomes monolayer and reduced the liposome size. The size of the OAChP liposomes was reduced by 30 nm as compared with the unmodified OA liposome. Results revealed that the anchored ChPs can improve the integrity and rigidity of the OA liposome.

1. Introduction

The surface of cells is rich in carbohydrate moieties that are attached to both membrane glycolipids and glycoproteins (Dicorleto and De La Motte, 1989). This carbohydrate-rich layer is known as glycocalyx (Palte and Raines, 2012). Glycocalyx contains a high amount of polysaccharide and is involved in cellular adhesion, intercellular communication, and biological recognition (Abeygunawardana and Bush, 1991; Sihorkar and Vyas, 2001). Therefore, the potential of polysaccharides to serve as ligand in the preparation of site-targeted liposomes has received wide attention (e.g. Mufamadi et al., 2011; Sihorkar and Vyas, 2001; Sunamoto et al., 1992). Among polysaccharides, the combination of chitosan with liposome has been a promising approach in the gene and drug delivery systems (e.g. Liu et al., 2011; Parabaharan, 2008; Riva et al., 2011; Zaru et al., 2009). Chitosan has been extensively used in the drug delivery system due to its biocompatibility, biodegradability, non-toxic nature, and cost effectiveness (e.g. Illum, 1998; Sheng et al., 2009). However, the use of chitosan in the biomedical field is often restricted by its poor solubility in water. Chitosan only dissolves in acidic condition (Chan et al., 2007). Previous studies have suggested that the solubility of chitosan in neutral and high pH condition can be improved by reducing its molecular weight (MW) (Illum, 1998; Kubota et al., 2000). The solubility of chitosan also can be improved by introducing the hydrophobic moiety at the primary amino group of the chitosan through acylation (Illum, 1998; Lee et al., 2005; Mourya et al., 2010; Ortona et al., 2008). The presence of hydrophobic moieties at the backbone of the chitosan can destroy the crystalline structure of chitosan and improve the solubility of chitosan in aqueous solution (Ge et al., 2007; Wu et al., 2006).

The hydrophobized chitosan can integrate with the lipid bilayer membrane as well as be used to prepare polysaccharide-anchored liposomes. The coating of hydrophobized chitosan on the surface of the liposome can enhance the liposome stability, structural rigidity, and membrane integrity (Ge et al., 2007). The preparation of chitosan-coated liposome with hydrophobized chitosan has its advantage compared with the unmodified chitosan. The hydrophobic moieties of the hydrophobized chitosan such as the palmitoyl group can be anchored onto the monolayer (outer layer) of the liposome bilayer through hydrophobic interactions (Wang et al., 2010). This method can prevent the desorption of the coated hydrophobized chitosan from the surface of liposome during storage and transportation (Sunamoto and Iwamoto, 1986; Sihorkar and Vyas, 2001).

So far, research conducted on the hydrophobized chitosan-anchored liposomes has been largely focused on the phospholipid-based liposomes (e.g. Garg et al., 2007; Ge et al., 2007; Wang et al., 2010, 2012; Qu et al., 2012), and the study on non-phospholipid liposomes has not been reported. There is continuous interest in the development of the non-phospholipid liposomes due to the disadvantages of phospholipids, such as reactivity in the

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biological milieu and high production cost (Gupta et al., 1996; Bastiat et al., 2007). Non-phospholipid liposomes can be prepared from several amphiphiles such as fatty acids (Bastiat et al., 2007) and the derivatives of polymers (Battaglia and Ryan, 2009; Discher and Eisenberg, 2002). In this study, the main objective is to prepare hydrophobized chitosan-anchored non-phospholipid liposomes using water-soluble *N*-palmitoyl chitosans (ChPs) with different DA. OA was selected as a model for non-phospholipid liposomes.

2. Methods and materials

2.1. Materials

All solutions and samples were prepared by using deionized water with a resistivity of 18.2 Ω/cm, from Barnstead Diamond Nanopure water Purification unit coupled with a Barnstead Diamond^{TM} RO unit (Barnstead International, USA). GC grade OA (99%), palmitoyl chloride, and acetic acid (99%) were purchased from Sigma (USA). Boric acid, hydrochloric acid (HCl), potassium bromide, and acetone were obtained from Merck (Germany). Sodium hydroxide (NaOH) and sodium nitrite ($NaNO_2$) were purchased from Fluka (Switzerland). Deuterated acetic acid (CD3COOD) and water (D_2O) were purchased from Armar Chemicals (Switzerland). Chitosan with an average molecular weight (MW_{avg}) of 150 kDa was obtained from Acros Organics (USA) and used as received.

2.2. Sample preparation

2.2.1. Preparation of water-soluble chitosan

The preparation of water-soluble chitosan was reported in previous works (Tan and Misran, in press). Briefly, 1% (w/v) of chitosan (MW_{avg} = 150 kDa) in 1% of acetic acid solution was prepared. 7 ml of 0.10 M of $NaNO_2$ was added dropwise into the chitosan solution under mechanical stirring. Then, the reaction mixture was stirred for 1 h. After that, the pH of the reaction mixture was adjusted to 8–9 in order to precipitate the undissolved chitosan. These undissolved chitosans were removed by filtration. The filtrate was neutralized to pH 7, and the remaining water soluble chitosan was precipitated by adding acetone. The precipitate was collected by using the centrifugation method at 5000 rpm for 2 min at 25 °C. The collected precipitate was washed with chloroform and dried overnight under vacuum.

2.2.2. Preparation of N-palmitoyl chitosans (ChPs)

Two types of ChPs with different DA (ChP1 and ChP2) were prepared from the water-soluble chitosan. First, 1% (w/v) of water-soluble chitosan solution was prepared. Then, the pH of the chitosan solution was adjusted to 7 using 1 M of NaOH. 27 μl and 55 μl of palmitoyl chloride were added to the chitosan solution under magnetic stirring at room temperature to prepare ChP1 and ChP2, respectively. After 5 h, the mixtures were neutralized. ChP1 and ChP2 were precipitated using acetone. ChP1 and ChP2 were collected by the centrifugation method at 5000 rpm for 2 min. ChP1 and ChP2 were washed repeatedly with chloroform to eliminate free fatty acid. Finally, the products were dried overnight under vacuum.

2.2.3. Preparation of ChP-coated OA liposomes

30 mM of OA liposome solution was prepared by dissolving an appropriate amount of OA in 50 mM of borate buffer (pH 8.8). ChP1 and ChP2 solutions with their concentration ranging from 0.05 to 0.30% (w/w) were prepared by dissolving an appropriate amount of ChP1 and ChP2 into 50 mM of borate buffer (pH 8.8). Then, the pH of OA liposome, ChP1 and ChP2 solutions was adjusted to 7 using 1 M of HCl. The liposome solution was added dropwise into ChP1 and ChP2 solutions individually under magnetic stirring. These mixtures were stirred for 24 h at room temperature (25 °C). Then, the pH of the mixtures was adjusted to pH 8.8 for the formation of ChP-coated liposomes, OAChP1, and OAChP2. These liposome solutions were incubated at 25 °C for 24 h before analysis.

2.3. Characterization of ChP1, ChP2, and liposome solutions

2.3.1. Average molecular weight determination

The average molecular weight (MW) of chitosan was determined by the Static Light Scattering (SLS) method using a Malvern NanoSeries ZetaSizer (UK) (Wu et al., 1995).

2.3.2. Fourier-transform infrared (FT-IR) spectroscopy

FT-IR spectra for chitosan, ChP1, and ChP2 in the form of KBr disc were obtained using the Perkin Elmer spectrometer (model RX-1, USA). Water-soluble chitosan, ChP1, and ChP2 were mixed with KBr (1:100) and compressed into pellets before analysis. The resolution of the IR spectra was 4 cm^{-1} and was recorded in 8 accumulations from 400 to 4000 cm^{-1} in the transmittance mode. The DA of ChP1 and ChP2 was calculated from FT-IR spectra using the following equation (Kasaai, 2008):

$$DA(\%) = \left(\frac{A_{1655}}{A_{3450}}\right) \times \frac{100}{1.33}$$

A_{1655} and A_{3450} are the intensity of the peak at 1655 cm^{-1} and 3450 cm^{-1} in FT-IR spectra, respectively.

2.4. ^{1}H NMR spectroscopy

The sample for ^{1}H NMR analysis was prepared by dissolving 10 mg of chitosan and ChP into 1 ml of 2% of CD_3COOD in D_2O solution. ^{1}H NMR spectra for all samples were acquired using a JEOL JNM-GSX 270 FT NMR spectrometer (270 MHz) at 20 °C.

2.5. Estimation of chitosan solubility

The solubility of water-soluble chitosan, ChP1, and ChP2 in water was determined using a UV–Vis spectrophotometer. First, a series of the water-soluble chitosan, ChP1, and ChP2 solutions with different concentrations was prepared. Calibration curves for the water-soluble chitosan, ChP1, and ChP2 were constructed by plotting the intensity of the absorption peak at wavelength 274 nm versus the concentration. Saturated chitosan, ChP1, and ChP2 solutions were then prepared, and their UV–Vis absorption was determined. The solubility of the chitosan, ChP1, and ChP2 solutions was estimated by the extrapolation method of the calibration curve.

2.6. Size and zeta potential

The size and zeta potential of the prepared liposomes were measured using a Malvern NanoSeries ZetaSizer (Malvern, UK) at a constant temperature of 25 °C. Size and zeta potential of the prepared liposome were monitored over 30 days of storage time.

2.7. Optical polarizing microscope imaging (OPM)

The OPM micrographs of the prepared liposomes were captured using a Leica Polarizing Microscope equipped with a Leica QWin software. All measurements were performed at a temperature of 25 °C.