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Control over micro-fluidity of liposomal membranes by hybridizing metal nanoparticles

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ABSTRACT

This study introduces a facile method to hybridize metal nanoparticles with lipid vesicles, which allows us to control over their membrane micro-fluidity. We have fabricated these hybrid liposomes by directly hybridizing metal nanoparticles with lipid bilayers solely consisting of 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC). For this, we have used the dehydration and rehydration method. Characterizing their morphology and micro-fluidity, in which we have used electron microscopy and fluorescence anisotropy spectroscopy, enables us to demonstrate that metal nanoparticles with different surface properties create interactions with either phosphorus end groups or hydrophobic tails of DPPC, thereby resulting in decrease in micro-fluidity of the assembled lipid membranes, especially for the hydrophobic layers. Our approach to hybridize metal nanoparticles in between lipid layers offers a flexible means that allows us to obtain a liposome system with more controllable membrane properties.

1. Introduction

Liposomes self-assemble to spontaneously form bilayers in water, eventually resulting in a unique lipid membrane shell structure; hydrophilic head groups of phospholipids form the inner and outer membrane surface, while their two hydrophobic tails form the hydrophobic phase of the membrane [1,2]. Due mainly to their cell membrane-like features, to date, they have gained great interests in researches on developing drug delivery systems that cannot only effectively encapsulate active molecules, such as drugs, but also controllably transport them through the lipid membrane. The transport rate of the actives encapsulated in the liposome is indeed important and usually determined by its membrane property. Therefore, a variety of techniques have been developed for controlled release [3–6]. Fluidity of the lipid membrane plays a critical role in providing high enough dispersion stability in complex formulations of foods, pharmaceuticals, and cosmetics, while controlling the release kinetics of encapsulates. However, it is known that conventional liposomes are relatively weak to endure a certain external stress, such as osmotic pressure, thereby easily breaking off their structure [7,8]. This is because they consist of an extremely thin lipid layer with weak interactions. To solve this problem, thus creating structurally more stable liposomes, new techniques, including curvature control [9–11], chemical reaction [12,13], and hybridization [14,15], are being developed. More recently, it has been reported that stable liposomes can be obtained by incorporating nanoparticles, such as surface-functional polymer nanoparticles (NPs) [16,17] and quantum dots [18]. This means that phase property changes have been induced in the vicinity of NPs. Using NPs is quite advantageous since it is truly flexible for fabricating a variety of liposomes. However, to better understand how NPs interact with lipid molecules, we need to more systematically characterize the phase property of lipid membranes.

In the present contribution, we introduce a facile method for fabrication of liposomes with controllable membrane micro-fluidity, in which metal NPs are hybridized with lipid molecules. We utilize the dehydration and rehydration technique that allows us to introduce metal NPs in between lipid layers of the membrane shell. Basically, these metal NPs have different surface properties; we have introduced either negative charges or polymer chains to generate the interactions with lipid molecules. Using these metal NPs enables us to show how they play a role in tuning the phase property of the lipid membrane. In this study, we confirm the morphology of hybridized liposomes by using electron microscopes. We

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also observe their micro-fluidity by using a fluorescence anisotropy technique [19–21]. This technique is useful because we cannot only determine the transition temperature of lipid membranes, but also characterize their fluidity with the concentration of metal NPs. For this, we incorporate fluorescence probe molecules into hydrophilic and hydrophobic layers, respectively, and analyze their mobility in the presence of metal NPs, thus characterizing the micro-fluidity for each layer.

2. Experimental

2.1. Materials

1,2-Dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC, PC 99.0%) was purchased from Doosan Biotech (Korea) and used as received. We always used double-distilled water, whose pH was adjusted to 7.4 by dissolving phosphate buffered saline (PBS) tablets (Sigma). $H_2PtCl_6{\cdot}xH_2O$ ($x = 5.6$) was purchased from Kojima Chemicals and used as a platinum (Pt) source. $HAuCl_4$ was also purchased from Aldrich and used as a gold (Au) source. Polyvinylpyrrolidone (PVP K15, Mw = 1.0×10^4 g mol^{-1}, Junsei) was used as a nucleation-prompting agent and stabilizer for Pt NPs. 1,6-Diphenyl-1,3,5-hexatriene (DPH) and 8-anilino-1-naphthalene sulfonate ammonium salt (ANS) were purchased from Aldrich.

2.2. Synthesis of metal nanoparticles

Au NPs were synthesized by using the chemical reduction method [22]. To synthesize Au NPs, we first dissolved $HAuCl_4$ (2.5 g) and PVP K15 (2 wt%) in a 200 mL round-bottomed flask filled with deionized water (100 mL). After adding sodium citrate (10 g) into the precursor solution, the reaction mixture was refluxed for 20 min at 100 °C. The diameter of Au NPs, synthesized using this method, was ~3.7 nm. Their ξ-potential was detected at −33 mV. We also synthesized Pt NPs by using the alcohol reduction method [23]. First, we completely dissolved $H_2PtCl_6{\cdot}xH_2O$ ($x = 5.6$, 0.1 g) in a mixture of ethanol/water (195.1 g) containing PVP K15 (2 wt%). The mixing ratio of ethanol to water was set to 1/1 (w/w). Then, the solution was heated to 75 °C for 4 h with reflux, eventually producing Pt NPs with ~2.4 nm by diameter. The size of metal nanoparticles was determined by directly analyzing the transmission electron microscope (TEM) images. After removing ethanol by evaporation, the volume was recovered to initial volume by adding water. ξ-potential

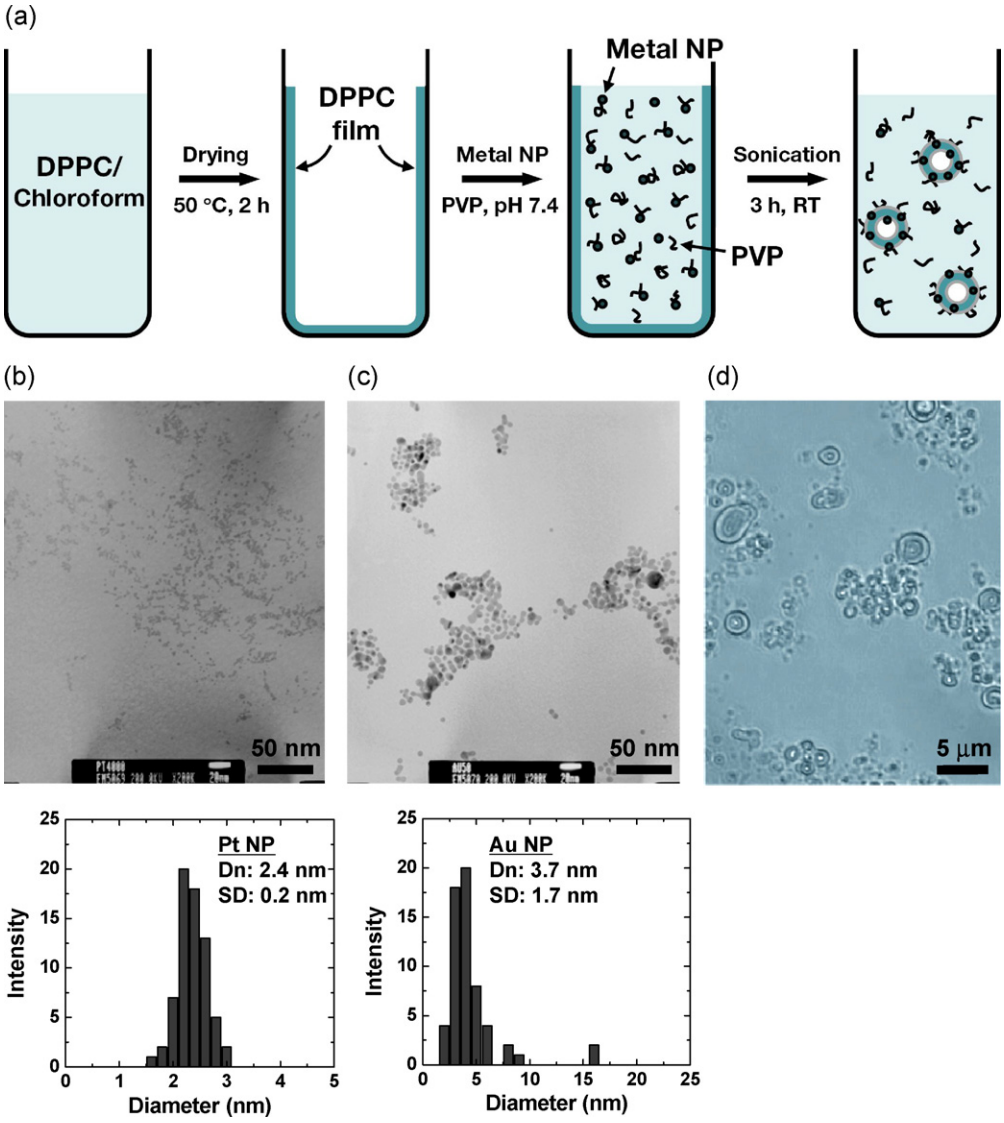

Fig. 1. The process for hybridizing metal NPs with DPPC liposomes. (a) Schematic presentation for the preparation of metal NPs-containing liposomes. (b) A TEM image and a size distribution of Pt NPs. (c) A TEM image and a size distribution of Au NPs. (d) Pt-loaded liposomes with single voids in the center produced right after the sonication. The image of the sample was observed by using a bright field microscope (Optiphot-2, Nikon). This sample contained approximately 70 ppm Pt NPs.