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Short communication

Anionic liposome template synthesis of raspberry-like hollow silica particle under ambient conditions with basic catalyst

Haruyuki Ishii, Kumi Sato, Daisuke Nagao, Mikio Konno*

Department of Chemical Engineering, Graduate School of Engineering, Tohoku University, 6-6-07 Aoba, Aramaki-aza Aoba-ku, Sendai, 980-8579, Japan

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ABSTRACT

Hollow silica particle was obtained with a vesicle template synthesis in water under ambient conditions in the presence of ammonia. Biomimetic vesicles, liposomes were used, which consisted of a zwitterionic phospholipid, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), and a tiny amount of charged amphiphiles, hexadecylamine (HDA) or dicetylphosphate (DCP). Aggregation of silica occurred for DPPC or cationic DPPC/HDA liposome, whereas well-dispersed hollow silica particle could be obtained for anionic DPPC/DCP liposome. The hollow particle synthesized with the anionic liposome had single-layered and raspberry-like structures. Electrostatic repulsion between anionic vesicles maintained stable dispersion of the as-synthesized particles during the reaction. Formation of the raspberry-like morphology is explained by silica particle precipitation selectively induced around the liposomes under basic conditions due to affinity of silica precursors for the liposomes. Synthesis of well-dispersed hollow silica particle with a raspberry-like morphology is the first report in vesicle template syntheses.

1. Introduction

Increasing attention has been paid to vesicle template syntheses of inorganic materials due to direct formation of unique architectures based on the lamellar structures of vesicle [1,2]. In recent years, the template syntheses of hollow inorganic particles have intensively been studied because the vesicles templates can simply be removed under mild conditions around ambient temperatures with neither damaging the hollow structures nor using hazardous solvent [3]. This feature is a great advantage of the syntheses over conventional hard-template syntheses, which require high temperature annealing or use of hazardous solvent for removal of the templates.

Among inorganic substances, special interest was shown in hollow silica particles [4–11], which encapsulated fluorescent marker [8,12], contained functional nanoparticles [11,13] or had surface modified with biomimetic lipid layers [12] designed for application to biotechnology and nano-reactor. The vesicle template syntheses have been used for other inorganic hollow particles, which were composed of palladium [3], copper oxide [14], or alloy metals [15]. In spite of the development of the vesicle template synthesis, particle aggregation and deficit formation are still problems which limit application of vesicle template synthesis. To overcome the problems and to further develop advanced applications, it is important to understand the mechanism of shell formation that should be affected by spatial distributions of reactant components, ionic species and electric potential in the vicinity of vesicle surface.

In silica biomineralization occurring in multicellular or unicellular organism, the products have multiple morphologies and hierarchical structures [16–18]. The reaction is often performed in water at the ambient temperature. Because intracellular vesicles involve the constructions of their diverse materials [16], the vesicle template syntheses have also been studied to mimic the biomineralization. The template syntheses of hollow silica particles were conducted not only in water at the ambient temperature [4,6,8,9] or accompanied with hydrothermal treatment [19–21] but also in water–alcohol mixture with basic catalyst [11,22]. In spite of differences in the reaction conditions, the products mostly have smooth silica surfaces.

Liposomes are artificially biomimetic vesicles composed of lipid bilayer. The present work examines liposome-template synthesis of hollow silica particle. In the aqueous liposome-template syntheses, high dispersion stability of liposome throughout the reaction is required for obtaining well-dispersed hollow particles without aggregation among the particles and collapse of the structures. Although polymeric vesicle templates can be stabilized in solution by both steric effects and surface charge, contribution of steric factors to stabilization of liposome templates is significantly low in comparison with that of polymeric ones. Since the introduction of surface charge is important for the improvement of the stability, charged liposomes are examined for the template synthesis of hollow silica particles. The syntheses are carried out in water with

* Corresponding author. Tel.: +81 22 795 7239; fax: +81 22 795 7241.
E-mail address: konno@mickey.che.tohoku.ac.jp (M. Konno).

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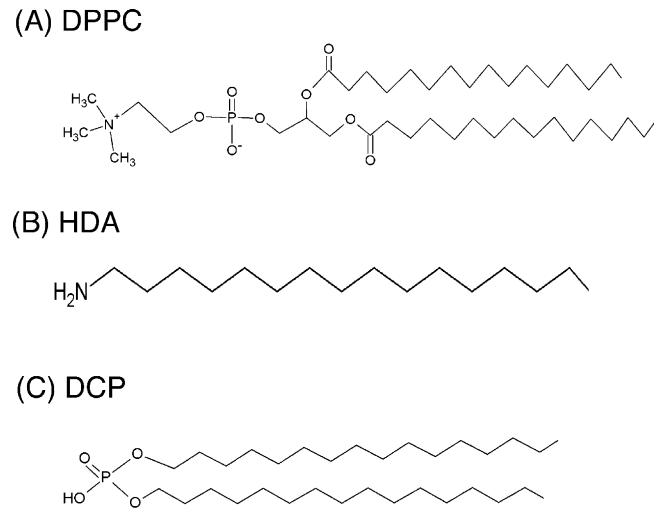

Fig. 1. Chemical structures of components used for the preparation of liposomes. (A) 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), (B) hexadecylamine (HDA), and (C) dicetylphosphate (DCP).

basic catalyst for promoting silica shell formation in sol–gel reaction [23]. This method may be able to shorten synthetic time that was typically several days long in the conventional aqueous syntheses of lipid or surfactant vesicle templates [4,6,8]. The silica shell formation is discussed in relation with surface properties and dispersion stability of the charged vesicles and with base-catalyzed sol–gel reaction in the vicinity of the liposomes.

2. Materials and methods

2.1. Materials

Liposomes were prepared with DPPC (NOF Corporation, Tokyo, JAPAN), DCP and HDA (Sigma–Aldrich, St. Louis, MO, USA). These molecular structures are shown in Fig. 1. Tetraethylorthosilicate (TEOS) and the other chemicals of analytical grade were purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.2. Liposome preparation

The extrusion technique reported elsewhere [24,25] was applied to the preparation of liposomes. In brief, three kinds of lipid solutions, DPPC, DPPC/DCP (99/1 mol%), and DPPC/HDA (98.9/1.1 mol%) dissolved in a chloroform–methanol mixture (2/1, v/v) at a concentration of 5 mg/mL, were put into a 100 ml round-bottom flask, followed by solvent removal by rotary evaporation under reduced pressure. The dried lipids were re-dissolved in the chloroform–methanol mixture and the solvent was evaporated again to obtain a homogeneous thin lipid film. The obtained lipid films were kept under high vacuum and then hydrated with water at 55 °C to form multilamellar vesicles (MLV). After five freezing–thawing treatments, the liposome size was reduced by extrusion of the MLV suspension through polycarbonate membranes with 100 nm pores placed in a Mini-extruder (Avanti Polar Lipids, Alabaster, AL, USA).

2.3. Base-catalyzed sol–gel reaction via hydrolysis and condensation of a silica precursor in the presence of three kinds of liposomes

Silica precipitation in the suspension of these liposomes was performed in ammonia aqueous solution without alcohols. TEOS was mixed with 0.1 mM liposome suspension and then ammonia was added to the mixture. Molar ratio of TEOS/DPPC/NH_3 was 80/1/100. All the experiments were performed at 35 °C below the phase transition temperature of DPPC (41 °C) because the formation of hollow silica particle without collapse of the structures is preferable to the use of vesicle with closely packed and solid-like gel phase [4,19]. After 24 h, the reaction mixture was centrifuged for removing soluble silica precursors, and then washed by ethanol–water mixture (50/50, v/v).

2.4. Characterization of silica products and liposome suspensions

The silica product obtained for each liposome suspension was observed with scanning transmission electron microscopy (STEM, HD2700, Hitachi High Technology, Tokyo, Japan). More than 100 diameters of hollow silica particles were measured to determine the size distribution and to calculate the averaged diameter. Zeta potentials of the liposomes were measured with ELS-8000 (Otsuka Electronics, Osaka, Japan) equipped with an apparatus for DLS. The DLS was used to measure dispersion size distributions of the liposomes in water and an aqueous NH_3 solution.

3. Results and discussion

3.1. Formation of silica products in the presence of three kinds of liposome

Fig. 2(A) and (B) show the silica products obtained in the liposome template syntheses using that composed of DPPC and DPPC/HDA, respectively. In the reaction with the DPPC liposome (Fig. 2(A)), reaction mixture was cloudy around 24 h after ammonia addition, and the product was silica aggregates that had irregular shapes and looked composed of hollow structures. The product with the cationic DPPC/HDA liposome in Fig. 2(B) was aggregates of hollow particles that were almost spherical. After the ammonia addition, the reaction mixture was transparent until 4 h, and then gave rise to white aggregates. The average diameter of the hollow spheres was ca. 100 nm that corresponded to the extruder pore size. These results suggested that the silica shell formation proceeded until 4 h on the DPPC/HDA liposome stably dispersed in the solution. In the reaction of Fig. 2(C) where anionic DCP was used instead of the cationic HDA, hollow particles were successfully obtained without aggregation. The average size of the particles measured with TEM was 111 nm ± 16 nm (±S.D.). Since the particle size distribution was well consistent with the dispersion size distribution of the reaction mixture measured with DLS (Fig. 2(D)), it was confirmed that the particles were well dispersed and did not form aggregation in the reaction. A highly magnified SEM picture in Fig. 3 (A) clearly indicates that the silica shell had raspberry-like morphology. The single layered and hollow structures of as-synthesized particles were confirmed from a broken profile in Fig. 3(B). The raspberry-like shell formation have been reported with the template syntheses using emulsion [26] or polymer particles with hairy polymer shell [27]. Although raspberry-like morphology has been reported in a vesicle template synthesis [10], this is due to interaction between small and large hollow silica particles with smooth shell. Therefore, the structure is different from the presently observed one that is the first report in vesicle template syntheses to our best knowledge.

3.2. Dispersion stability and surface charge of liposomes under reaction conditions

DLS and ELS analyses were performed to study the structures and surface properties of the liposomes used in the present work.