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Fabrication and characterization of pseudo-ceramide-based liposomal membranes

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ABSTRACT

We present a facile and straightforward method to fabricate liposomal membranes with a significantly stable lamellar structure consisting of pseudo-ceramide, fatty acid, and cholesterol. Characterizing their membrane properties, in which we have used differential scanning calorimetry, X-ray diffraction, and FT-IR spectra, enables us to demonstrate that pseudo-ceramide with appropriate amounts of stearic acid and cholesterol can assemble to form a stable lamellar α-phase. Moreover, we show that cholesterol is indeed important and plays a role in controlling the melting entropy of lipid membranes, which is attributed to a disordered molecular packing, thus creating more flexible liposomal membranes. This approach to use pseudo-ceramide offers a useful means to fabricate a variety of biocompatible liposomes with controllable membrane properties, which enlarges their applicability in the field of drug delivery, dermatology, and cosmetics.

1. Introduction

Stratum corneum, the outermost layer of skin, is known to provide an epidermal barrier against trans-epidermal water loss as well as environmental irritants [1,2]; for example, removing stratum corneum drastically increases the epidermal water loss by approximately 100-fold [3,4]. This barrier function originates from a unique hierarchical structure of lipid-rich intercellular matrix; highly ordered lipid lamellae, typically filling in the intercellular matrix, can provide stratum corneum with physicochemical barrier properties, which is truly important in maintaining internal water homeostasis. A lipid lamellar phase of stratum corneum is basically made up of a variety of components, including ceramides, cholesterol, cholesteryl esters, fatty acids, etc. [5–9]. Among them, ceramides play critical role in both forming lipid bilayers and generating structural stability of resulting lamellar phase, which is closely related to the control over the mechanical properties, such as diffusivity and elasticity.

In nature, unfortunately, ceramides exist only in an extremely small quantity. Moreover, it is really difficult to extract them with high purity in large production scales, which hampers their wider use in industry. To make them commercially available, synthetic pseudo-ceramides have been developed and used to reduce transdermal water loss, thus moisturizing the skin in dermatological and cosmetic applications [10–13]. They have been found to display almost similar functions to natural ceramides, when applied to dried or damaged skins; when a pseudo-ceramide is applied to the skin, it also exhibits an excellent ability to retain water therein. Furthermore, when it is formulated with other components, such as cholesterol and fatty acid, we can obtain better barrier properties. This is due to its high affinity to the intercellular lipid lamellar phase as well as ability to assemble to form a lamellar phase [14,15].

When pseudo-ceramide is formulated with fatty acid and cholesterol, the mixed phase is equilibrated to form a membrane, a thin lipid film [16,17]. Although this membrane can be either parallel or curved, its physical property usually depends on its chemical composition. In principle, by characterizing its physical property, we can build up a stable membrane system, while controlling the permeability of encapsulated or composite ingredients. In the viewpoint of fabricating the structurally stable lipid membrane, hybridizing pseudo-ceramide with other basic lipid components may create molecular interactions in between them, which usually improves the mechanical strength of resulting membrane. This is truly advantageous since we may achieve flexibility in tuning both membrane rigidity and permeability. From the standpoint of performance and applicability, therefore, we need to figure out how

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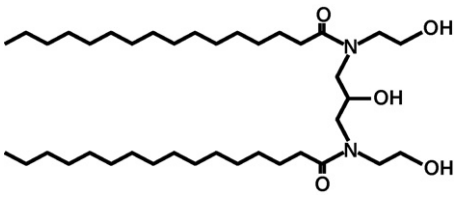

Scheme 1. A molecular scheme of ceramide PC104.

pseudo-ceramide has an influence in changing phase property of lipid membranes, thus widening its practical applicability in industry.

In this contribution, we introduce a facile method for fabrication of liposomes, whose membrane consists of pseudo-ceramide, cholesterol, and fatty acid. We use ceramide PC104 (1,3-bis-(*N*-(2-hydroxyethyl)-palmitoylamino)-2-hydroxypropane (Scheme 1)) which is one of well known pseudo-ceramide [18–20]. Basically, we demonstrate that utilizing the dehydration and rehydration technique, in which we use both proper combination and concentration of those three components, enables fabrication of stable liposome dispersions. To better understand how they generate, we characterize their membrane properties by measuring crystallinity and transition temperatures. For this, in this study, we confirm the phase property of lipid films by using differential scanning calorimetry, X-ray diffraction, and FT-IR spectra, which allows us to demonstrate how the pseudo-ceramide interacts with other molecules, thereby leading to controllable phase patterns. Finally, we show the pseudo-ceramide is also applicable for fabricating both liposomes and lipid lamellar structures.

2. Experimental methods

In a typical fabrication procedure, we used the dehydration and rehydration method to prepare ceramide-based liposomes [21,22], as shown in Fig. 1A; we first dissolved ceramide PC104 (1,3-bis-(*N*-(2-hydroxyethyl)-palmitoylamino)-2-hydroxypropane, Macrocare Co., Korea), stearic acid (Sigma), and cholesterol (Sigma) in a solvent mixture of chloroform and methanol (9/1, v/v) in a glass round flask and then completely evaporated the solvent to dryness by using a rotary evaporator, thus obtaining a thin lipid film on the inner surface of the flask. After drying process, we hydrated the lipid film with a PBS buffer solution, whose pH is 7.4, at 80 °C while swirling for 5 min. After hydration, we applied sonication to the hydrated lipid film for 1 h, thus generating ceramide-based liposomes. The lipid concentration in final formulations was set to 80 mg mL^{-1}. The size of liposome particles was characterized by using light scattering (Malvern 3000HSA).

To characterize the property of lipid membranes, we first prepared the lipid samples in glass test tubes with a Teflon-sealed screw cap and then melted them at ~100 °C, which provides a homogeneous phase mixing. Then, the samples were cooled to form lipid thin films. Molecular structural information of ceramide PC104 in the thin films was observed by measuring the extent of molecular interactions between amido groups with a FT-IR spectrometer (Mangna-IR 760, Nicolet). Measuring the absorption wavelength of C=O stretching vibration of ceramide PC104, which is directly associated with its C–N stretching allows us to determine the degree of molecular interactions, typically caused by hydrogen bonding. The structure and state of the lipid films were identified by measurement of X-ray diffraction with a small angle X-ray diffractometer (Bruker) in the 2θ range to 30°. We used Cu Kα radiation ($\lambda = 1.542$ Å) in these measurements. The d-spacing (Å) was converted from the diffraction angles (θ) by using the Bragg equation: $n\lambda = 2d \sin\theta$. Thermal properties of the lipid films were observed by using a differential scanning calorimetry (DSC Q1000, TA Instrument); first we loaded an appropriate amount of film sample onto a high volume pan. Then we scanned temperature from −20 °C to 180 °C with a rate of 2 °C min^{-1} under nitrogen flow. Melting enthalpy, ΔH_m was determined by integrating the area under the curve from a plot of the excess heat capacity as a function of temperature. Melting entropy, ΔS_m was calculated from $\Delta S_m = \Delta H_m / T_m$.

3. Results and discussion

Ceramide, stearic acid, and cholesterol are known as key components that consist of inter-cellular lipid lamella in stratum corneum layer. To better provide their flexible applicability, in this study, we have fabricated liposomes consisting of ceramide PC104, stearic acid, and cholesterol by using the dehydration and rehydration method. We have observed that using these three components that range at weight fractions of 0.2–0.55, 0.3–0.65, and 0.1–0.3 enables us to obtain stable liposomes, as shown in Fig. 1B. Once liposomes have generated after applying sonication, they are very stable for long time, which is confirmed by observing the changes in liposome sizes by using light scattering. In an optimum composition, the initial particle size and dispersion stability have remained unchanged for more than several months at room temperature. The particle size usually ranges from tens of nanometers to hundreds of nanometers. Most of liposomes have a unilamellar wall structure, which is a common observation when we use the dehydration and rehydration method [23,24]. Since we have set up a basic system to develop liposomes with ceramide PC104, stearic acid, and cholesterol, our interest have moved to if we can exactly characterize the phase property of liposomal membranes.

Basically, we have tried to figure out how ceramide PC104 plays a role in changing phase property of the lipid membranes,

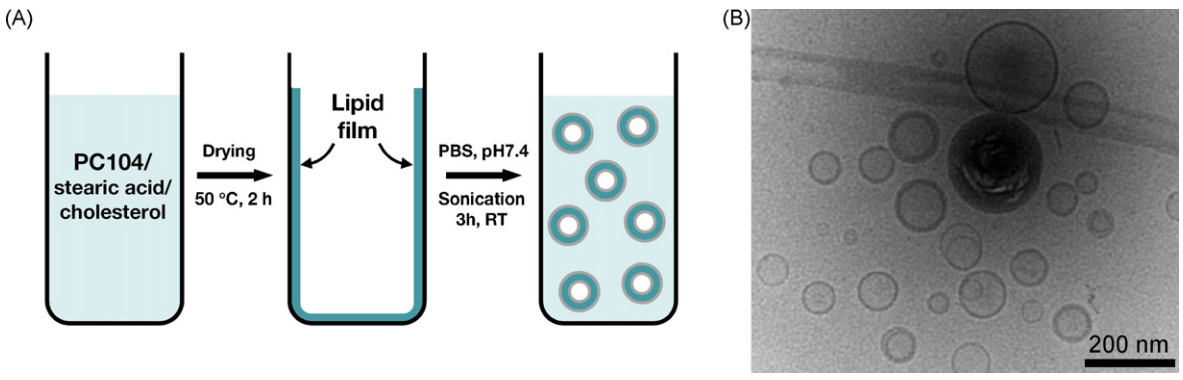

Fig. 1. (A) Schematic for the preparation of liposomes by using the dehydration and rehydration method. (B) Cryo-TEM micrograph of liposome composed of ceramide PC104, cholesterol and stearic acid. The dispersion was prepared at the weight ratio ceramide PC104/stearic acid/cholesterol = 40:40:20 (w/w/w).