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Nanoemulsification of pseudo-ceramide by molecular association with mannosylerythritol lipid

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ABSTRACT

Ceramide molecules in water-based solutions readily attract each other to form molecular crystals, which seriously hampers to diversify their formulations. This paper describes a facile method that allows fabrication of stable ceramide emulsions through an effective molecular association with a lipid having an asymmetric molecular geometry. The lipid considered in this study is mannosylerythritol lipid (MEL). MEL is specialized in having a unique molecular structure containing sugar alcohol erythritol as a hydrophilic part and two alkyl chains with different number of carbons as hydrophobic moieties. Our particular interest has been focused on experimentally demonstrating how MEL interacts with pseudo-ceramide molecules by observing phase properties, emulsion morphology, and suspension stability. The pseudo-ceramide emulsions prepared with MEL show remarkably improved dispersion stability without either formation of molecular crystals or changes in particle sizes even after storing them for a long time. This suggests that MEL readily associates with the pseudo-ceramide due to the hydrophobic interaction, while it makes a break in the continuity of the molecular assembly of the pseudo-ceramide molecules themselves due to the geometric hindrance coming from MEL's asymmetric molecular structure.

1. Introduction

Stratum corneum (SC) is an essential element for the skin, since it displays the barrier function and regulates the trans-epidermal water loss [1–4]. In the absence of SC, trans-epidermal water loss increases approximately 100-fold [5]. This performance is originated from the complex intercellular structure of the SC layer. Intercellular lipids exist with a form of highly aligned lamellae. This lamellar is composed of ceramides, free fatty acids, cholesterols, *etc.* Ceramides take ~0.33 mole fraction of the intercellular lamellar composition [6,7]. Typically, ceramides consist of a polar amide group and nonpolar alkyl chains and display an excellent ability to assemble to form the lamellae due to their intermolecular interaction between alkyl chains. This makes them highly desirable for dermatological applications [8–10]. However, it is difficult to directly fractionate natural ceramides from living tissues or organs because of extremely low yields. Hence, pseudo-ceramides, such as 1,3-bis-(N-(2-hydroxyethyl)-palmitoylamino)-2-hydroxypropane (PC-104, Scheme 1) [11–14] and N-(3-hexadecyloxy-2-hydroxypropyl)-N-2-hydroxyethyl hexadecanamide (SLE) [15,16], having similar molecular properties, have been developed. However, despite their excellent performance in the skin barrier function, pseudo-ceramides are likely to form molecular crystals even less than 1% by mass in most of formulations. This is because pseudo-ceramides exist either as γ-crystal in the frozen state or α-crystal in the melt state [17–19]. The α-crystal phase gradually returns to the γ-crystal phase, as it is metastable.

Lipid molecules can co-assemble with ceramide derivatives. Currently most of commercialized lipids are synthetic ones. As alternates, recently, new types of lipids and lipid derivatives have been bio-friendly produced by employing microbial lipid fermentation technologies [20–22]. They are known to remarkably improve the ecological compatibility by lowering the potential toxicity, which makes them more widely applicable for foods, pharmaceutical and cosmetic applications [23–25]. A representative example is mannosylerythritol lipid (MEL, Scheme 2) [26–28]. It is a microbial extracellular glycolipid, produced from *Candida* sp. *SY16*, one yeast strain. MEL has a molecular geometry consisting of 4-*O*-β-D-mannopyranosyl-*meso*-erythritol and a fatty acid and/or an acetyl group, respectively. Basically, MEL cannot only reduce the interfacial tension at liquid–liquid interfaces to form stable emulsions or to remove contaminants, but also show the anti-microbial performance. It is featured by the two hydrophobic alkyl chains with

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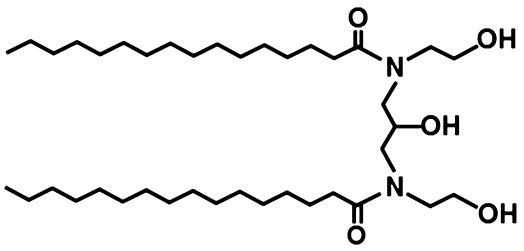


Scheme 1. The molecular structure of PC-104.

different carbon numbers, thus giving rise to an asymmetric molecular geometry. When this MEL is mixed with the ceramide, the mixture is expected to show a different type of molecular associations, which will be essential for controlling the phase properties of complex formulations containing ceramide.

The ultimate goal of this paper is to figure out how MEL associates with pseudo-ceramide, PC-104. On the basis of this, we try to fabricate extremely stable pseudo-ceramide based nanoemulsions. The phase properties were characterized through X-ray diffraction and differential scanning calorimetry. The particle morphology was observed *via* bright field and polarized optical microscopy and transmission electron microscopy. The long-term emulsion stability was examined by measuring the particle size and zeta-potential changes. Finally, we show that MEL is important and plays a role in regulating the molecular crystallization of pseudo-ceramide molecules in complex formulations.

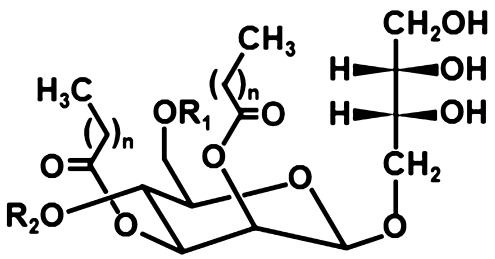
2. Experimental

2.1. Materials

Pseudo-ceramide (1,3-bis-(N-(2-hydroxyethyl)-palmitoyl-amino)-2-hydroxypropane, PC-104) was kindly supplied from Macrocare (Korea). Mannosylerythritol lipids (MEL) was received from Damy Chemical (Korea). Sodium N-lauroyl-L-glutamate (LS11, Ajinomoto, USA) was used as a surfactant. For all experiments, deionized double distilled water was used.

2.2. Preparation of emulsions

In order to prepare emulsions, first, PC-104 was completely melted in MEL at 60 °C. Then 1 wt% LS11 aqueous solution was poured into the mixture at 60 °C. Pre-emulsions prepared by applying sonication in a bath for 10 min were re-emulsified for 1 min at room temperature by using a probe-type sonicator (VCX130, Sonic & Materials Inc., US). This emulsification procedure produced fine emulsions. To observe the effect of solid content, we changed the oil concentration from 0.5 wt% to 20 wt%. For this case, the mixing ratio of MEL to PC-104 was set to 5–5 by weight. To fine the optimized mixing ratio for achieving stable emulsions, MEL concentration was also controlled against PC-104.



Scheme 2. The molecular structure of MEL.

2.3. Analysis of phase properties

The crystallinity of PC-104 after mixing with MEL was identified with an X-ray diffractometer (XRD, Rigaku, Japan) equipped with a two-theta/theta scanning mode, a 40 kV/100 mA X-ray, and a RINT 2000 wide angle goniometer. In the measurement, 2θ was varied from 30° to 60°. The d-spacing was considered based on the diffraction angles (θ) by using the Bragg equation: $n\lambda = 2d\sin\theta$, where d is the lattice spacing, 2θ is the scattering angle, and λ is the wavelength of the X-ray beam. The thermal property of PC104/MEL mixtures was measured by using a differential scanning calorimetry (DSC, Universal V4.3A, TA Instrument, USA). The generation of hydrogen bonding in the mixture of PC-104/MEL was observed by measuring the extent of molecular interactions between amido groups with a FT-IR spectrometer (Mangna-IR 760, Nicolet). For DSC analysis, the test sample was loaded onto a high volume pan. The heating rate was adjusted to 5 °C/min in the range from −40 °C to 100 °C under the nitrogen flow. Then, the melting temperature for each sample was assigned by determining the heat flow.

2.4. Determination of particle sizes

The particle size and zeta potential of emulsions were characterized with a dynamic light scattering (ELS-Z, Otsuka electronics, Japan). The excitation light source was a 10 mW He–Ne laser at 632.8 nm and the intensity of the scattered light was measured at 173°. The zeta potential was calculated from μ_E using the Smoluchowski equation. To avoid any interference during the measurement, the exact test volume, which was 1 ml, was taken and injected into the zeta potential cell. Each measurement was repeated four times and the average was taken.

2.5. Microscopic observations

Emulsion drops in the micrometer range were observed with a bright field microscope and their crystallinity was characterized with a polarized optical microscope (NSB-80T, Samwon, Korea). The morphology of nanoemulsions was analyzed with a translation electron microscope (TEM, Energy-Filtering Transmission Electron Microscope, LIBRA 120, Carl Zeiss, Germany). The acceleration voltage was 120 kV. A drop of the sample was put on a 400 mesh carbon coated copper grid (Ted Pella, Inc.). The test sample was negatively stained by using a 1 wt% uranyl acetate solution. Then, the stained sample was completely dried in air before TEM observation.

3. Results and discussion

3.1. Intermolecular interaction of pseudo-ceramide

The molecular character of pseudo-ceramide, PC-104, is analogous with that of natural ceramides. One of key features is their ability to have a strong hydrophobic interaction between themselves in most of solvent systems. In principle, this interaction force leads to formation of the γ crystal phase. To experimentally show this unique behavior, a demonstration study was conducted. 5 wt% of PC-104 was dissolved in oil at 80 °C. We have observed that on cooling the solution, PC-104 was separated from the oil and formed a crystal-like phase, as shown in Fig. 1. This phenomenon could be commonly observed even in emulsion formulations prepared with the aid of surfactants [13]. The hydrophilicity of PC-104 is relatively weak, as it has just three hydroxyl groups. Two long alky chains have the same number of carbons. This molecular geometry favors generation of the strong hydrophobic interaction between alky chains, resulting in macroscopic molecular crystallization in any formulations. Because of this, even though PC-104 holds many benefits for skin applications, its incorporation into a variety of