Biomimicking of intercellular lamellar phase using new types of ceramides, 1-*O*-stearoyl ceramide NP and ceramide EOP with ultra-long chain (C24-C32)

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Abstract

Background: In this study, we investigated how 1-*O*-stearoyl ceramide NP (1OS Cer) and ceramide EOP with ultra-long chain (C24-C32) (ULC Cer) associate with ceramide NP (CerNP), respectively, by characterizing their assembed 2-D ceramide-based lipid membrane (CLM) structures.

Methods: For this, a CLM of stratum corneum (SC) composed of ceramides/free fatty acid/cholesterol was fabricated by using the Langmuir-Blodgett (LB) 2-D assembly technology.

Results: The π-A isotherm of the CLMs showed that the ULC Cer led to the formation of a packed molecular-membrane, whereas the 1OS Cer hindered the molecular association of CLMS. Moreover, the 1OS Cer impeded phase transition compared with the ULC Cer, which was confirmed by fluorescence microscopy. Based on these results, we determined the surface roughness of CLMs after transfer them onto the mica substrate through atomic force microscopic observation. These results revealed that the ULC Cer had heterogeneous phase in monolayer than phase containing the 1OS Cer.

Conclusion: We proved the novel properties of the ceramide types, 1OS Cer and ULC Cer. The notion from this study can definitely explain the functions of new ceramides in the 3D-lamellar structure that is the actual SC model as well as in monolayer.

Keywords: ceramides; lipid membranes; monolayer; stratum corneum

Introduction

Stratum corneum(SC), the outer layer of skin barrier, plays an important role in preventing epidermal water loss as well as protecting the skin against pathogens. It has been well known that the ceramide plays the most critical role in determining the skin barrier function in the intercellular lipid phase of SC [1]. The main lipids in SC are ceramide (Cer), cholesterol (Chol) and free fatty acid. These three lipids exist in an approximately equal molar ratio. Of three lipid classes, the Cer is most important, which occupies about 40 wt% in human SC. Generally, Cer consists of a sphingoid base as a backbone with an amide linked one acyl chain. Also Cers have lots of species of more than 15 classes [2]. Cers are classified with the types of the fatty acid and the sphingoid base. For instance, the most abundant species of Cers is CerNP, with a non-hydroxylated fatty acid (N) linked to phytosphingosine with a 4-OH (P). And CerNH constructed with a non-hydroxylated fatty acid (N) linked 6-hydroxy-sphingosine with a 6-OH and 4,5-double bond (H) is also well known.

Universally, the molecular area per lipid and the formation of monolayer are studied by the LB technology [3]. Actually there are so many studies for noted Cers such as Cer NS and CerNP in the LB system, and (S) in CerNS means sphingosine with a 4,5-double bond. CerNS with short acyl chain have a higher area by surface pressure-area isotherm and show more conventional phase separation in the AFM image [4]. And many studies demonstrated CerNP in monolayer formed only the liquid-condensed film at the air/water interface and phase transition between the liquid-expanded and liquid-condensed films was not observed [5].

In this study, we investigated how various types of Cers associate with SC-lipids, CerNP/Chol/Stearic acid (SA) by LB system. The new types of Cers, the ULC Cer and the 1OS Cer containing acyl chains in both N- and 1-O-position [6], which are recently developed, are used in our study. We performed analyses of fluorescence microscopy and AFM to visualize the domain of CLMs to verify properties for each structure of Cers.

Materials and Methods.

Materials

Ceramide NP (C16) was supplied by Dusan(Korea). Stearic acid, sodium hexadecyl sulfate and chloroform were purchased from Sigma-Aldrich(USA). Cholesterol was obtained from Amore-Pacific (Korea). Deionized double-distilled water (DI water) was used for all experiments.

Formation of CLMs at the air-water interface

Molecular assembly of SC lipids was investigated using the LB deposition. The SC lipids with equal molar ratio were dissolved in chloroform (1 mg/ml). We prepared the three types of sample, a mixture of basic SC lipid containing CerNP, Chol, stearic acid (SA) (mixSC). And the mixSCs with the ULC Cer or the 1OS Cer were named to mixSC$_{ULC\alpha}$ or mixSC$_{1OS\alpha}$, (α : weight fraction of ULC Cer or 1OS Cer) which the sum of weight fraction of the types of Cers in a sample is 1. Subsequently, the solution of mixSCs was spread onto the water phase and allowed to evaporate for 15min. The spread lipid phase was compressed at 10 mm/min at the room temperature by closing and opening the barriers. The surface pressure was measured by using a Wilhelmy plate at least in triplicate. The lipid membrane at the air-water interface was compressed to 45 mN/m of surface pressure.

Surface topology observation CLMs via fluorescence images and AFM

The CLMs at the air-water interface were transferred onto a mica substrate by raising the mica support vertically through the air-water interface at 1 mm/min. By using this transferred substrate, fluorescence images of CLMs dyed with texas red and morphology through AFM observation were obtained.

Results

In order to investigate how the new types of Cers associate with CerNP in the CLMs, we measured the π-A isotherm at air-water interface using three types of mixSCs (Figure 1a). As we mentioned above, what the phase transition of the CLMs containing Cers is unapparent. The π-A isotherm of the mixSC showed a rapid phase transition in around 130 cm^2. As the ULC Cer was added in increasing from 5 wt% to 20 wt% (mixSC$_{ULC0.05\sim0.2}$), the molecular area versus same surface pressure was compressed (Figure 1b). So that, it

revealed the ULC Cer help to form a closely packed membrane. However, the molecular area of the CLM was increased in equal surface pressure, when the 1OS Cer was added in mixSC increasing weight fraction from 0.05 to 0.2 (mixSC$_{1OS0.05\sim0.2}$) (Figure 1c). That meant the 1OS Cer retarded the assembled the CLM at air-water interface due to its parallel structure. The cycled π-A isotherm, hysteresis experiment, showed that the area in contrast to hysteresis of mixSC$_{1OS0.2}$ was declined in high rate than other types of Cers, although the degree of changed hysteresis was low at all three types of compositions (Figure 1d-f).

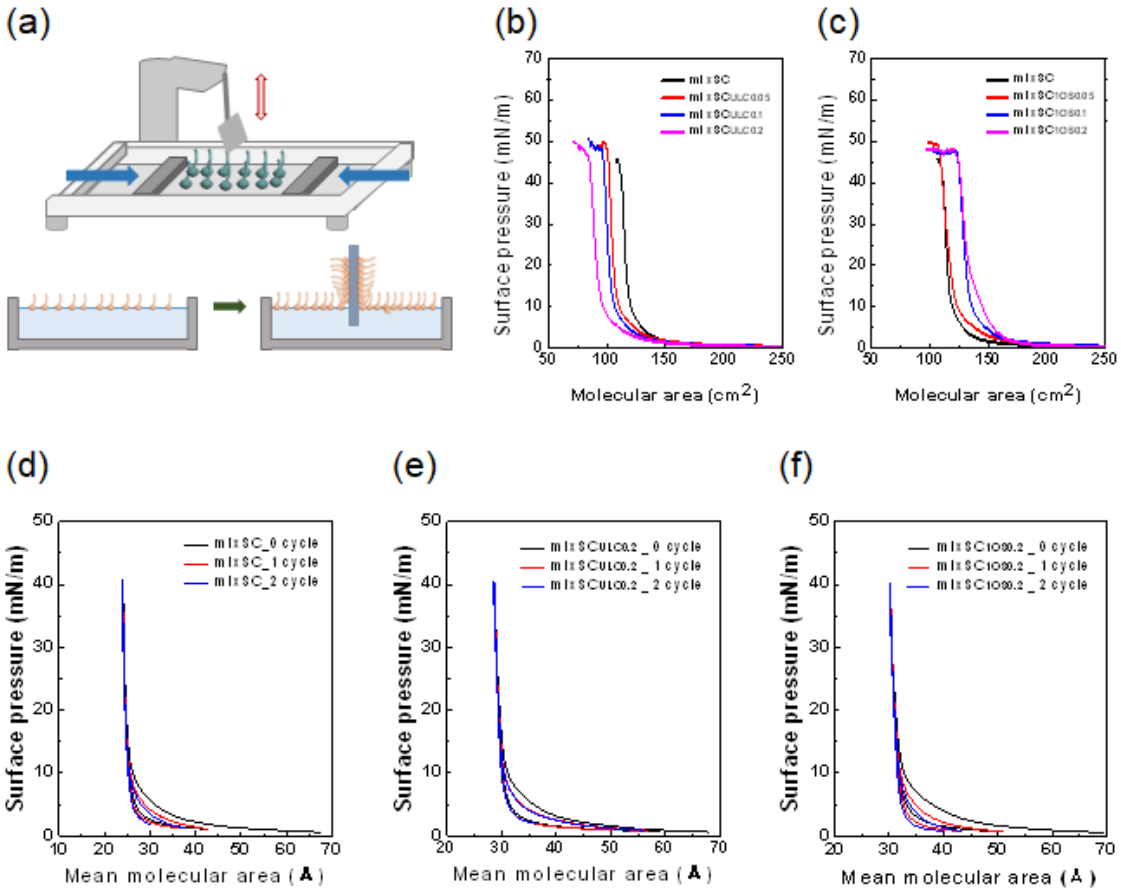

Figure 1. (a) Schematic illustration of a Langmuir trough process. (b) Surface pressure – area isotherms of mixSC (100 ~ 80 wt% Cer NP) with 0 ~ 20 wt% of ULC Cer or (c) 1OS Cer. Isocycle traces of (d) mixSC in the third cycles, (e) mixSC$_{ULC0.2}$ and (f) mixSC$_{1OS0.2}$.

To identify the phase transition of the films of CLMs, we obtained the fluorescence images of mixSCs depending on surface pressure (10 mN/m, 13 mN/m, 15 mN/m, 20 mN/m, 25 mN/m, 45 mN/m). Fluorescence dye we used was Texas red-DHPE. For the film of mixSC, the phase transition was rapidly increased at 20 mN/m. And compared to other types of films, the molecular area was ambiguous even at 45 mN/m, the plateau region of solid phase (Figure 2a). Then, we observed that the phase transition from liquid to solid occurs at a surface pressure as low as 15 mN/m in mixSC$_{ULC0.2}$. The molecular areas were distinct above 20 mN/m (Figure 2b). So, the ULC Cer caused the faster phase transition due to its strong hydrophobic interaction. It is very interesting to compare it with the 1OS Cer, which induces a phase transition at a relatively high surface pressure. Also, the molecular associated structures and their z-height caused by the structural impact of the 1OS Cer could be clearly confirmed in the CLM of mixSC$_{1OS0.2}$ (Figure 2c).

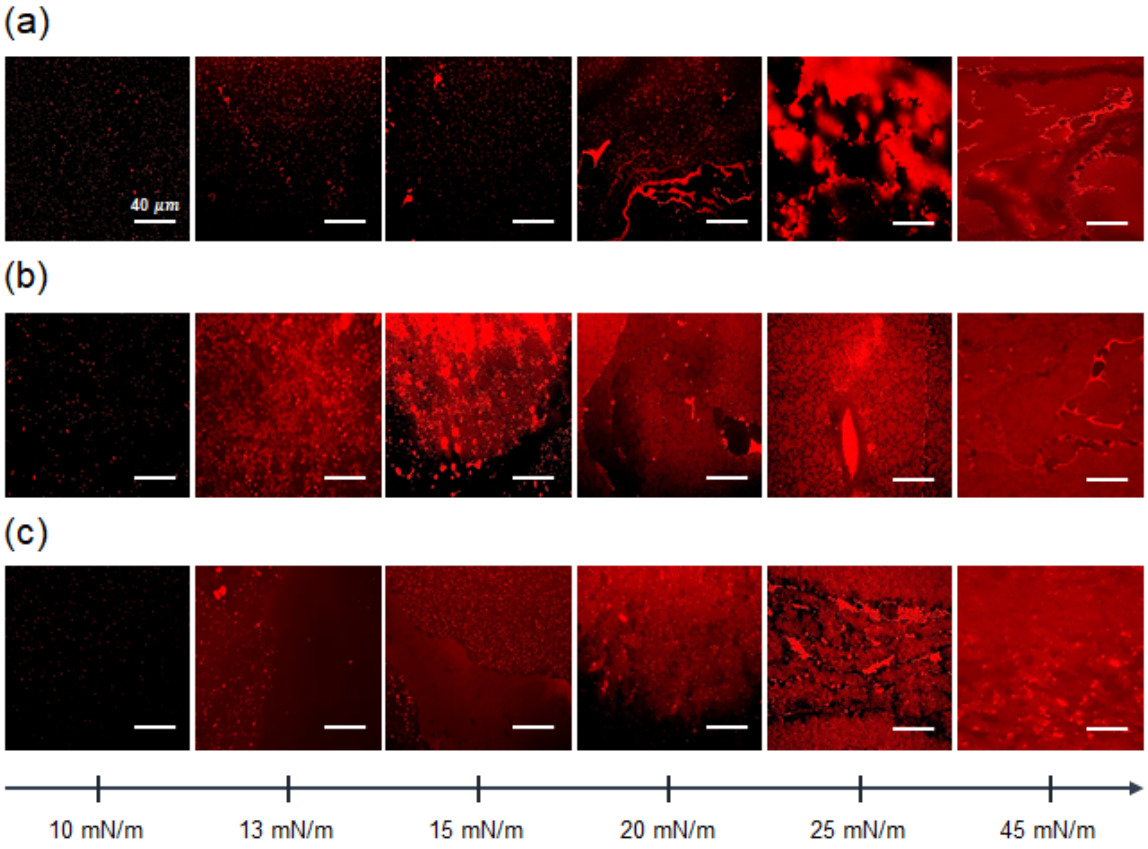

Figure 2. Fluorescence microscopy images of CLMs depending on surface pressure, (a) mixSC, (b) mixSC$_{ULC0.2}$ and (c) mixSC$_{1OS0.2}$.

In order to confirm the effect of presence of new Cer types, we measured the topology of CLMs on mica substrate by using AFM. The scan size was 20 μ*m*. Although all three types of Cers had low and similar roughness at the same scan area, the roughness of the film of mixSC containing only CerNP was relatively lowest. Considering from the point of view of molecular assembly behavior, the film of the mixSC had a considerable homogeneous phase over a wide range (Figure 3a). However, film of the mixSC$_{ULC0.2}$ presented a heterogenous phase appearing to various areas which mean small molecular area, compared to the mixSC (Figure 3b). In the composition of mixSC$_{10S0.2}$, it showed an obvious gap of z-height over the scan region owing to the position of the hydrophobic acyl chain of the 1OS Cer at the air-water interface (Figure 3c). Consequently, these results demonstrated that the ULC Cer affects the formation of the closely packed CLMs than the 1OS Cer which forms a disordered CLM over a wide area.

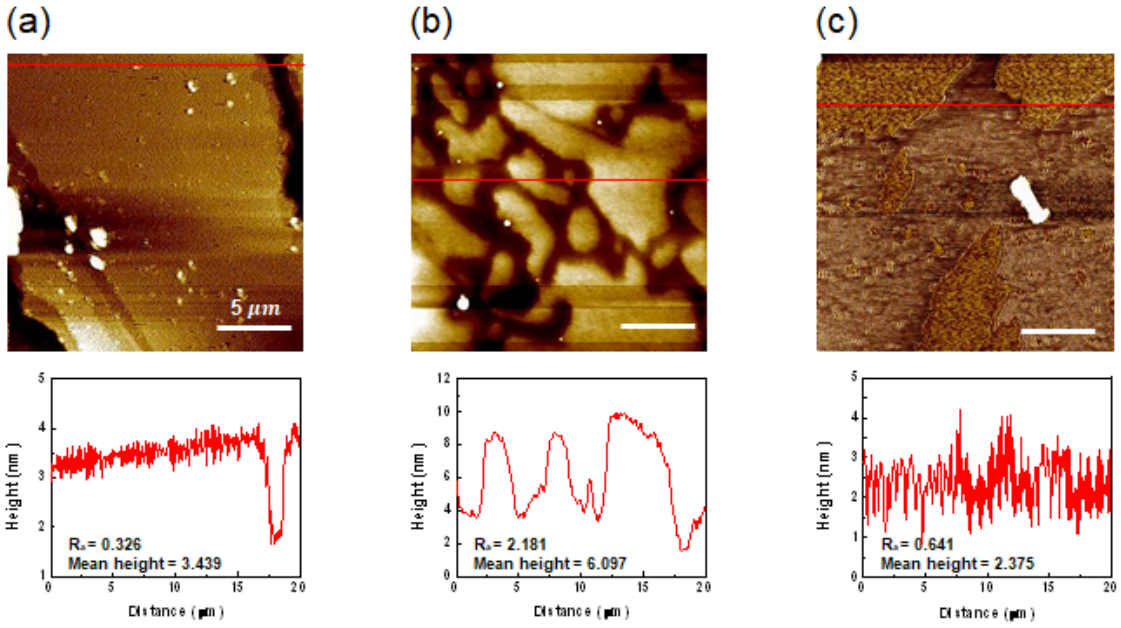

Figure 3. Effect of the presence of the ULC Cer and the 1OS Cer on the organization of CLMs. AFM images obtained for (A) mixSC, (b) mixSC$_{ULC0.2}$, (c) mixSC$_{1OS0.2}$.

Discussion

This research is the first attempt to analysis of that novel Cers. So that, we propose a CLM system in which novel Cers, 1OS Cer and ULC Cer, were associated with CerNP, Chol, SA. Because of the structure of the 1OS Cer, the CLM containing the 1OS Cer is expected to form a expanded and disordered phase. However, we predicted that the ULC Cer would affect the powerful interaction of hydrophobic chains caused by ultra-long chain (C24-C32) combined with backbone. Further studies on what functions of the 1OS Cer and the ULC Cer were critical for formation of such a intercellular lamellar layer and how it affects the skin barrier function would allow us to achieve great technological advances in the field of skin care.

Conclusion

The asymmetric acyl chains of the 1OS Cer enabled occupation more area per one molecule during membrane formation, thereby impeding tight molecular association of the lipid membrane. While the ULC Cer led to formation of a closely packed lipid membrane due to their strong hydrophobic interaction. Furthermore, the incorporation of the 1OS Cer slowed down the phase transition compared with the case of the ULC Cer in the same surface pressure. These results highlight that the new Cers, 1OS Cer and ULC Cer directly affect the formation of the lipid lamellar phase, which is closel related to the regulation of skin barrier function.

Acknowledgments

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Conflict of Interest Statement.

NONE.

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