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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Introduction:

Stratum corneum (SC), 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 Langmuir Blodgett (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 ceramide-based lipid membranes (CLM) to verify properties for each structure of Cers.

Materials & Methods:

Preparation of CLMs aligned 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 mixSCULCα or mixSC1OSα, (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

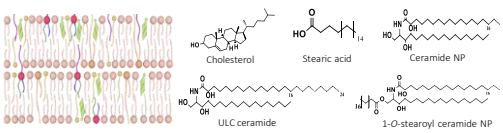
The CLMs at the air-water interface were transferred onto a mica substrate by raising the mica support vertically through the air-water interface at 1 mm/min. By using this transferred substrate, fluorescence images of CLMs dyed with texas red and morphology through AFM observation were obtained.

Formation of CLMs containing surfactants

Each CLMs was fabricated at the air-water interface plotting the π-A isotherm. The π-A isotherm was shifted as compression and expansion were repeated several times by one sample. However, the gap between shifted isotherms seemed to be neglectable at specific time. At that times, the barriers were reopened and the arranged CLAs were expanded and dispersed. The another surfactant, CTAB, was added to each CLM by increasing the weight fraction of surfactants in contrast to the proportion of the ceramides for observing intensity of the CLMs.

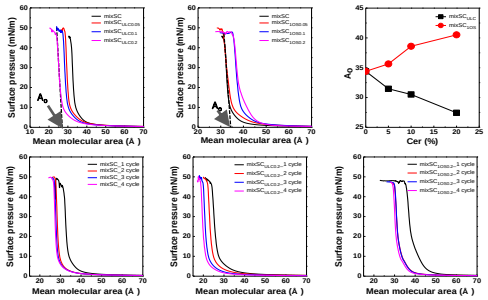
Results & Discussion:

Stratum corneum and lipid Molecular structures



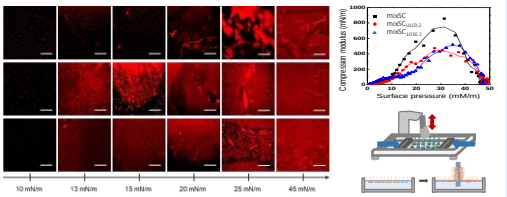
Results & Discussion:

Π-A isotherm of the mixSCs



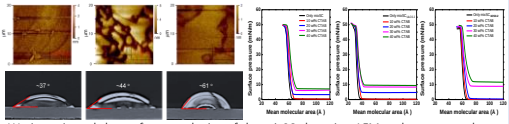
We performed the p-A isotherm for each mixSC at the air/water interface. As the results, the ULC Cer led to formation of the tightly densed membrane, while the 1OS Cer disturbed the alignment of the membrane.

Comfirm the phase transition for each type of ceramide



The scale bar of all fluorescence image is 40 um. We visually checked the phase transition of the membrane at the specific surface pressure. The ULC Cer showed the most rapid phase transition.

Characterization of the 2-D membranes



We investigated the surface topologies of the mixSCs by using AFM and contact angle. The tilted phase of the mixSC$_{1OS0.2}$ induced the most hydrophobic membrane. In addition, we verified that the association with the molecules of ULC Cer and CerNP make the membrane closely packed, by adding cationic surfactant on the membranes of the mixSCs.

Conclusions:

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.

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References:

1.Smedan J.V, Bouwstra J.A. (2016) Stratum Corneum Lipids: Their Role for the Skin Barrier Function in Healthy Subjects and Atopic Dermatitis Patients. Curr Probl Dermatol. 49:8-26
2.Schmitt T, Neubert R H.H. (2018) State of the art in Stratum Corneum research: The biophysical properties of ceramides. Chem.Phys.Lipids. 216:91-103
3.Norlen L, Gil I.P, Simonsen A, Descouts P. (2007) Human stratum corneum lipid organization as observed by atomic force microscopy on Langmuir-Blodgett films. J.Struct.Biol. 158:386-400
4.Pullmannova P, Pavlikova L, Kovacik A, et al (2017) Permeability and microstructure of model stratum corneum lipid membranes containing ceramides with long (C16) and very long (C24) acyl chains. Biophys. Chem. 224:20-31
5.Imura T, Sakai H, Yokoyama S. et al (2000) Atomic force microscopic study on the surface properties of phospholipid monolayers containing Ceramide 3. Colloids Surf.B 19:81-87
6.Rabionet M, Bayerle A, Marsching C. et al (2013) 1-O-acylceramides are natural components of human and mouse epidermis. J.Lipid Res. 54:3312-3321