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Phase Separation in Lipid Lamellae Result from Ceramide Conformations and Lateral Packing Structure

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Intercellular lipids in the stratum corneum protect the living body from invasion by allergens and pathogens, and also suppresses water evaporation within the body. It is important to understand how differences in the microstructure of intercellular lipids arise. This microstructure is affected by lipid composition. Studies using intercellular lipid models have reported the formation of two phases with different short lamellar periodicities. However, the details of the packing structure characteristics of the two phases observed in these intercellular lipid models are unclear. Our previous report revealed that different short periodicity phases coexist in the N-(α-hydroxyoctadecanoyl)-dihydrosphingosine (CER[ADS]), cholesterol (CHOL), and palmitic acid (PA) complex model. In this study, the characteristics of the packing structure of two phases with different short lamellar periodicities, which were observed in the intercellular lipid model (CER[ADS]/CHOL/PA) that we used previously, were adjusted for models with different lipid compositions. The characteristics of the packed and lamellar structures have been determined by temperature-scanning small-angle X-ray scattering and wide-angle X-ray diffraction measurements simultaneously. These differences in lamellar structure were thought to be caused by differences in ceramides (CER) conformation between the hairpin and the V-shape type. The lamellar structure of the V-shaped CER conformation has a low orthorhombic ratio. The above results suggest that an increase in the ratio of CER with the V-shaped structure causes the lamellar structure to have low orthorhombic ratio, thereby contributing to a decrease in the bilayer’s barrier function.

Key words  stratum corneum; intercellular lipid; short lamellar; ceramide; X-ray diffraction

Introduction

The stratum corneum (SC) intercellular lipid not only protects the living body from invasion by allergens and pathogens but also suppresses water evaporation. Thus, it plays a pivotal role in the skin’s barrier function.1 Intercellular lipids are made up of ceramides (CER), cholesterol (CHOL), cholesterol esters and free fatty acids (FFAs).2

Intercellular lipids form a characteristic microstructure, comprising a short lamellar structure and a long lamellar structure with a repeating distance of approximately 6 and 13 nm, respectively.3 The lamellar period is inversely correlated with trans epidermal water loss (TEWL), a representative index of the skin’s barrier function.4,5 In fact, the lamellar period is shorter in patients with atop dermatitis than in healthy subjects.6 On the other hand, intercellular lipids of human SC have different packing structures of hydrocarbon chains, and it is known that orthorhombic (Orth) and hexagonal packing (Hex) and liquid crystals coexist as different substructures.7–9 The density of lateral hydrocarbon chain packing is decreased in the order of Orth, Hex, and liquid crystal.9

Increasing TEWL indicates deterioration of the SC’s barrier function. Some reports have indicated that differences in the hydrocarbon chain packing of intercellular lipids are also correlated with TEWL.4,7–9 However, it is difficult to elucidate the relationship between lipid composition and packing structure due to the intricate lipid composition of human intercellular lipids; there are 342 CER species, grouped into 12 subclasses on the basis of chain length and head group structure.9,10

Many studies were conducted to investigate the influence of lipid composition on hydrocarbon chain packing by mimicking the intercellular lipids of the human SC.11–20 In this context, some groups reported that two different CER conformations formed in distinct phases of lamellar periodicity.17–20

We previously reported that phases of different periodicity coexist in the N-(α-hydroxyoctadecanoyl)-dihydrosphingosine (CER[ADS]), CHOL, and palmitic acid (PA) complex model in which structural analysis was carried out using simultaneous small-angle X-ray scattering (SAXS) and wide-angle X-ray diffraction (WAXD) measurements at constant temperature.20 However, the details of the packing structure of the two phases observed in these intercellular lipid models are unclear. 2H solid-state NMR spectroscopy,17,18 neutron diffraction measurement, and X-ray diffraction measurement without temperature scanning, could not discriminate the differences in packing structure in phases with different lamellar periods.14,17–19

In order to determine the characteristics of hydrocarbon chain packing of each lamellar structure, we needed to conduct structural analysis using simultaneous SAXS and WAXD measurements.

The utility of structural analysis of intercellular lipids by simultaneous SAXS and WAXD measurements has previously been reported.21 We used intercellular lipid models (CER[ADS]/CHOL/PA) with different lipid compositions from what we have reported previously.14 We characterized the packed structure and the lamellar structures by analyzing

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the temperature-scanning simultaneous SAXS and WAXD measurements against two phases with different lamellar periodicities.

**Experimental**

**Materials** (2S,3R)-2-(2-Hydroxyhexadecanoyl)aminooctadecane-1,3-diol (95%): Ceramide [ADS]-C18/16(CER[ADS]) was supplied by Takasago International (Tokyo, Japan). Cholesterol (CHOL: 99%) and palmitic acid (PA: 99%) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Chlo- roform and methanol were certified grade. Other chemicals used were reagent grade.

**Lipid Model** The lipid composition of the bilayers of CER[ADS], CHOL, and PA selected centroids of three previously reported clusters. The centroids of each cluster (Fig. 1) are representative of the three lipid bilayer groups. In previous reports, clusters were classified into four different types; however, the focus of this study was CER[ADS]. Hence, we selected three models that tended to increase the proportion of CER[ADS] instead. The representative composition of the three models are summarized in Table 1.

The CER[ADS]/CHOL/PA bilayer in Model 1 contains a high molar ratio of PA. In contrast, the CER[ADS]/CHOL/PA bilayer in Model 3 contains a low molar ratio of PA. We observed that the ratio of PA decreases while the ratio of CER and CHOL increases from Model 1 to Model 3. We prepared our lipid model in accordance with the method of Bangham. The lipid mixture is shown in Table 1 was dissolved in chloroform:methanol (2:1, v/v). A rotary evaporator was later used to remove the solvent and obtain a thin lipid film, which was dried in a vacuum for about 24 h. In order to adjust the total lipid concentration to 10 mM in the eggplant flask, 10 mL of acetate buffer (pH 5.3) was added and the lipids hydrated for 30 min above the phase transition temperature (about 85 °C). After removing the film from the flask, it was subjected to ultrasonic liquid processor irradiation (VCX 130 PB, Sonics & Materials, Inc., Newtown, CT, U.S.A.) for 5 min above the phase transition temperature (about 85 °C). The CER[ADS]/CHOL/PA bilayer suspension was stored at room temperature overnight. The vesicle aggregates were suction-filtered and dried, and used as an intercellular lipid model.

**Simultaneous Small-Angle X-Ray Scattering and Wide-Angle X-Ray Diffraction Measurements** The SAXS and WAXD profiles of the CER[ADS]/CHOL/PA bilayers were obtained at the BL40B2 (Structural Biology II Beamline) in SPring-8 (Hyogo, Japan). The X-ray wavelength was 0.083 nm and the sample-to-detector distance was approximately 540 mm. The scattering intensity $I$ (in arbitrary units) was measured as a function of the scattering angle ($\theta$), from which the scattering vector ($q$) was calculated in reciprocal nm. The latter is defined as $q = (4\pi \sin \theta)/\lambda$, where $\lambda$ is the wavelength. From the positions of a series of equidistant peaks ($q_n$), the periodicity ($d$) of a lamellar phase was calculated using the equation $d = 2n \pi/q$, where $n$ is the order number of the scattering peak. When a characteristic peak position (reciprocal space) is related to the real space, we use the term spacing, which is equal to $2\pi/q$ at that peak position. The reciprocal spacing $q = (4\pi \sin \theta)/\lambda$ was calibrated from the lattice spacing ($d = 5.838$ nm; $d$ is the lamellar repeat distance) of a silver bhenenate crystal at room temperature. The exposure time was about 30 s. The diffraction pattern was circular averaged to obtain a radial intensity profile. A sample cell containing the CER[ADS]/CHOL/PA membrane was sealed with a polyimide film and placed in the sample holder of the X-ray diffractometer. The temperature of the sample was controlled between 25 to 85 °C using differential scanning calorimetry (FP-99; Mettler-Toledo, Tokyo, Japan), and the temperature was measured with a thermocouple embedded in the sample holder. All the experiments were performed with a heating scan at a rate of 0.83 K min$^{-1}$. Hence, the total exposure time was about 72 min in a single scan. The X-ray scattering profile was recorded every 2.5 °C.

**Data Analysis** We applied Lorentz functions to fit the SAXS profiles and Gaussian functions to fit the WAXD profiles.

**Table 1. Composition of Various CER[ADS] Containing Model Lipid Mixtures**

| Model | Composition and molar ratio (CER[ADS]/CHOL/PA) | Short lamellar phase A (25 °C) | Short lamellar phase B (25 °C) | Phase separated PA (25 °C) | Phase separated CHOL (25 °C) |
|-------|---------------------------------------------|-----------------------------|--------------------------------|--------------------------|--------------------------|
|       |                                             | Peak position (nm$^{-1}$)   | Repeat distance (nm)         | Peak position (nm$^{-1}$) | Repeat distance (nm)     |
| 1     | 17.8:5.5:76.6                              | $q_1 = 1.362, q_2 = 2.724, q_3 = 4.084$ | 4.61                          | $q_1 = 1.461, q_2 = 3.019, q_3 = 4.476$ | 4.30 | - | + | - |
| 2     | 26.5:13.9:59.6                             | $q_1 = 1.354, q_2 = 2.714, q_3 = 4.048$ | 4.64                          | $q_1 = 1.447, q_2 = 2.968, q_3 = 4.448$ | 4.34 | + | + | - |
| 3     | 36.6:42.8:20.6                             | $q_1 = 1.362, q_2 = 2.724, q_3 = 4.084$ | 4.61                          | $q_1 = 1.475, q_2 = 2.991, q_3 = 4.462$ | 4.26 | - | + | - |

* Lamellar organization with their repeat distances are shown.

**Fig. 1. CER[ADS], CHOL, and PA Models**

Closed circles indicate the lipid composition of each model.
rhombic hydrocarbon chain packing ($R_{\text{Hex/Orth}}$) was defined as:

$$R_{\text{Hex/Orth}} = \frac{(I_{15} - 2 \times I_{17})/3}{I_{17}}$$

Where $I_{15}$ is the intensity of the diffraction peak at $q = 15.0 \text{nm}^{-1}$, and $I_{17}$ is that of the peak at $q = 17.0 \text{nm}^{-1}$. The numerator indicates the apparent area derived from Orth and the numerator indicates the apparent area of Hex. $2 \times I_{17}$ means the apparent area derived from Orth in the area of the diffraction peak at $q = 17.0 \text{nm}^{-1}$. When Orth and Hex appear in the CER[ADS]/CHOL/PA bilayer at the equivalent ratio, the $R_{\text{Hex/Orth}}$ value is 1. Greater $R_{\text{Hex/Orth}}$ values indicate that Hex is dominant in the CER[ADS]/CHOL/PA bilayers.

In addition, scattering data were analyzed by generalized indirect Fourier transformation (GIFT) method software package. Using the thickness scattering parameter of a pair distance distribution function (PDDF), $P_r(q)$ corresponds to the convolution of the electron density profile of the monolayer. Hence, electron density profiles i.e., DECON profiles, $\Delta \rho(r)$, can be obtained via deconvolution of the PDDF. The DECON profile is the electron density profile for the lipid bilayer from the hydrophobic group terminal to the hydrophilic group terminal, including hydration water effects with the electron density of water as the zero ordinate, $\Delta \rho(r) = 0$.

Results

Membrane Properties and Microstructure of CER[ADS]/CHOL/PA Bilayers

We determined the different conformations of our CER[ADS]/CHOL/PA samples. SAXS gives information about ordered multi-lamellar structures and enables detection of lipid lamellar structures and corresponding repeat distances of various lipid membranes. WAXD gives information about the lateral packing present in lamellar structures. When the lipid alkyl chains are packed hexagonally, a singlet is observed at around $q = 15 \text{nm}^{-1}$ while lipid tails that partly adopt an Orth result in two peaks at approximately $q = 15$ and $17 \text{nm}^{-1}$. The corresponding scattering patterns of these lipid membranes are shown in Fig. 2.

The scattering patterns of Models 1 and 2 at $25^\circ C$ (Fig. 2(a)), are characterized by the presence of scattering peaks at about $q_1 \approx 1.3$, $q_2 \approx 2.7$, and $q_3 \approx 4.1 \text{nm}^{-1}$, with a repeat distance of about 4.6 nm. This pattern indicates the formation of the short periodicity phase (Phase A). However, besides the presence of Phase A, an additional phase was also observed. This additional phase is characterized by scattering peaks at about $q_4 \approx 1.4$, $q_5 \approx 3.0$, and $q_6 \approx 4.5 \text{nm}^{-1}$ with a repeat distance of about 4.3 nm, indicating the formation of another short periodicity phase (Phase B). The lamellar period of about 4.6 nm is shorter than the 6 nm period of the short lamellar structure observed in the human SC. This is due to the short chain length of CER[ADS]-C18/16 used in this study.

Phase separation of FFA and CHOL reportedly occurs in the intercellular lipid model. In pure crystals of PA, a scattering peak is observed at $q = 1.76 \text{nm}^{-1}$. In pure CHOL crystals, however, a scattering peak is observed at $q = 1.82 \text{nm}^{-1}$.

In the scattering patterns of the Models 1 and 2 (Fig. 2(a)), the peaks are positioned at $q = 1.76 \text{nm}^{-1}$, and the crystalline CHOL phase is separated. As-PA has a similar chain length to the fatty acid chain of CER[ADS]-C18/16, PA is particularly poorly compatible with CER[ADS], therefore, phase separation of PA is often observed.

In the scattering patterns of Models 2 and 3 (Fig. 2(a)), the peaks are positioned at $q = 1.82 \text{nm}^{-1}$, and the crystalline CHOL phase was separated. Other studies have reported that phase-separated CHOL does not affect the multilamellar lipid organization. In our SAXS profiles at $25^\circ C$, two peaks were observed at $q = 1.56 \text{nm}^{-1}$ and $q = 1.48 \text{nm}^{-1}$, indicating that Phases A and B coexist with crystalline PA and CHOL (Fig. 2(a)). In WAXD profiles of Models 1 and 2 at $25^\circ C$, two peaks are observed at $q = 15$ and $17 \text{nm}^{-1}$, indicating that Orth...
and Hex coexist (Fig. 2(b)). On the other hand, in Model 3 (Fig. 2(b)), no peak was observed at $q \approx 15$ and 17 nm$^{-1}$, but multiple diffraction peaks were observed at different positions around $q = 13.1$, 13.5, 13.9, and 15.3 nm$^{-1}$. Accordingly, Phase A and Phase B do not form Orth and Hex in Model 3. Table 1 summarizes the scattering peak position, repeat distance, and phase-separated crystals observed in each model. As indicated in Table 1, in the lipid model using CER[ADS]/CHOL/PA, we found that Phases A and B were observed at any lipid composition ratio. Table 2 summarizes the diffraction peak positions, lattice constant, peak intensities, sublattices of the diffraction peaks, $R_{\text{Hex/Orth}}$ observed in each model. In Table 2, the $R_{\text{Hex/Orth}}$ of Model 2 is higher than that of Model 1 i.e., Model 1 has the highest ratio of Orth.

Changes in X-Ray Profiles with Temperature The SAXS and WAXD data from each model are plotted against temperature. Fig. 3. SAXS and WAXD Profile of Typical CER[ADS]/CHOL/PA Bilayers Belonging to Each Model Monitored at 25–85°C

![Figure 3](image)

Table 2. Composition of Various CER[ADS] Containing Model Lipid Mixtures

| Model | Hexagonal (25°C) | Orthorhombic (25°C) | HEX/OR peak ratio* (25°C) |
|-------|-----------------|----------------------|--------------------------|
|       | Peak position (nm$^{-1}$) | Peak intensity (a.u.) | Peak position (nm$^{-1}$) | Peak intensity (a.u.) |                  |
| 1     | 15.274          | 8404.276             | 17.062                   | 1403.565              | 1.33              |
| 2     | 15.636          | 7284.696             | 17.420                   | 846.288               | 2.20              |
| 3     | —               | —                    | —                        | —                     | —                 |

Lipid lateral packing is shown. The HEX/OR peak ratio as determined by the gaussian peak fitting procedure of the WAXD profile at 25°C are also presented. *The abbreviations HEX and OR stand for hexagonal and orthorhombic lateral packing, respectively.
temperature in Fig. 3. To analyze the difference in packing structure between Phases A and B, the peaks around $q \approx 1.3, 1.4, 13.9, 15, \text{ and } 17 \text{ nm}^{-1}$ in the temperature range 25–85 °C, we plotted the profiles of intensity $I$ versus the reciprocal spacing $q$ in Fig. 4. Regarding selection of the diffraction peak with $q \approx 13.9 \text{ nm}^{-1}$, the diffraction peak with the highest intensity at 25°C was selected from among the multiple diffraction peaks shown in Fig. 3(f). For the peak intensity observed around $q \approx 15 \text{ nm}^{-1}$ ($I_{15}$), due to the diffraction derived from the Orth and Hex overlap, the value of $I_{Hex} ((I_{15} - 2 \times I_{17})/3)$ was used to analyze the diffraction only from Hex.

The peak intensity observed around $q \approx 1.36 \text{ nm}^{-1}$ ($I_{1.36}$) derived from Phase A, which is a short lamellar structure with a lamellar distance of about 4.6 nm, decreases from around 45 °C to around 65 °C with increasing temperature (Figs. 4(a) and (e), respectively). Corresponding to the thermal behavior of $I_{1.36}$, the peak intensity observed around $q \approx 15 \text{ nm}^{-1}$ ($I_{Hex}$) and $q \approx 17 \text{ nm}^{-1}$ ($I_{17}$) derived from Orth and Hex decreases from around 45 °C to around 65 °C (Figs. 4(b) and (d), respectively). In Fig. 5(a), the peak positions of the scattering peaks

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**Fig. 4.** Changes in X-Ray Scattering Peak Intensities of Typical CER[ADS]/CHOL/PA Bilayers Belonging to Each Model as a Function of Temperature (25–85 °C)

Data shown are SAXS profiles (left row) and WAXD profiles (right row) of Model 1 (a and b, respectively), Model 2 (c and d, respectively), and Model 3 (e and f, respectively). Numbers on the left figures represent parameters of intensity near $q \approx 1.3 \text{ nm}^{-1}$ and $q \approx 1.4 \text{ nm}^{-1}$, while those on the right represent parameters near $q \approx 13.9 \text{ nm}^{-1}$ ($I_{13.9}$), $q \approx 15 \text{ nm}^{-1}$ ($I_{Hex}$), and $q \approx 17 \text{ nm}^{-1}$ ($I_{17}$). In Model 3, the scattering peak observed at around $q \approx 1.49 \text{ nm}^{-1}$ is hidden by the strong scattering peak derived from the lamellar liquid crystal near $q \approx 1.54 \text{ nm}^{-1}$ at 45 °C and above.
The constituent lipids of Phase B are thought to be composed mainly of CER and CHOL. The diffraction peak observed at $q \approx 13.9\,\text{nm}^{-1}$ derived from Phase B has not been focused so far, but in the X-ray diffraction profile of human SC reported by Doucet et al., a diffraction peak was observed prominently around $q \approx 13.9\,\text{nm}^{-1}$ in the deep portion of the SC, suggesting that a packing structure similar to Phase B also forms in human SC. Bouwstra et al. mentioned that a 4.3 nm phase with a Phase B–like lamellar period was observed in a pilot study on the psoriasis scale in the human SC.

In Model 3 (Figs. 4(e) and (f)), the peak intensity observed around $q \approx 1.36\,\text{nm}^{-1}$ ($I_{13.9}$) derived from Phase A decreases from around 45 °C; however, the decrease in peak intensity is gradual from 55–65 °C, and the thermal behavior does not agree with $I_{13.9}$. Moreover, in Fig. 5(a), the lamellar period of the scattering peak observed around $q = 1.36\,\text{nm}^{-1}$ from Phase A shows that the lamellar period gradually increases from 25–75 °C until the peak disappears. In contrast to Models 1 and 2, there was no apparent shortening of the lamellar period associated with a phase transition. We observed that in Phase A of Model 3, lamellar liquid crystals were formed above 25 °C. In Model 3, where Phase A does not cause phase transition because it is a lamellar liquid crystal, an increase in intensity was not observed for $I_{1.3}$ and $I_{13.9}$ (Figs. 4(e) and (f)).

On the other hand, in small angle $I_{1.4}$ observed around $q = 1.49\,\text{nm}^{-1}$ derived from Phase B, the peak position was shifted to $q = 1.54\,\text{nm}^{-1}$ at 45 °C or higher, and as the peak position shifted to $q = 1.54\,\text{nm}^{-1}$, an increase in peak intensity was observed and a phase transition to lamellar liquid crystal was observed (Figs. 5(b) and 4(e)). It was difficult to analyze after 45 °C, but we assumed that the peak intensity of $I_{1.4}$ in Model 3 also decreased after 45 °C as in Models 1 and 2, and the thermal behavior corresponds to $I_{13.9}$.

We found that the packing structure formed in Phase A varies depending on the lipid composition, and that Phase B forms a crystal structure different from Orth and Hex in each model.

Electron Density Profile (DECON Profile) Figure 6 shows the calculated electron density profile for each sample. The DECON profile is the average of the data from Phases A and B. The DECON profile rose slowly, increasing from the hydrophobic portion toward the hydrophilic portion and converging on the zero value at $r = \approx \text{approx. 2.8 nm}$ (Fig. 6(a)). The region where the zero value is obtained is thought to coincide with the region where hydration water exists near the hydrophilic part. Since twice the zero value distance exceeds the lamellar periodicity, a hydration layer appears to have formed near the hydrophilic part of the lipid.

The electron density for Model 3 is higher than that for Models 1 and 2 (lower CHOL and CER concentrations) at distances of 1.7–2.2 nm from the hydrophobic group terminus. The electron density of CHOL is reportedly higher than that of hydrophobic chains and equal to that of water. Hence, this indicates that condensed CHOL is localized to the region from the middle part of the hydrophobic groups of intercellular lipids to the vicinity of their hydrophilic groups at distances ranging from 1.7–2.5 nm (Fig. 6(a)). Although it is not possible to determine the physical properties of the bilayer from the DECON profile alone, it can be inferred that the flexibility of the bilayer is high since CHOL is relatively abundant. These findings support the specificity of Model 3, where Phase A
forms lamellar liquid crystals above 25 °C, and in Phase B, the transition to the lamellar liquid crystal phase occurs at around 50 °C, compared with the other models.

Discussion

In this study, we observed Phase A and Phase B structures using an intercellular lipid model (Models 1–3) consisting of the three components, CER[ADS]/CHOL/PA. In interpreting the lamellar periodicity of Phase A, considering that the distance of all-trans C–C bonding is 0.127 nm, the maximum chain length of α-hydroxy fatty acid is 2.29 nm. The calculated length of the hairpin-shaped CER molecules facing each other within a bilayer membrane has a lamellar period of about 4.6 nm, which is the length of the alkyl chains and coincides with the lamellar period of Phase A.

Phase B is thought to correspond to a scattering peak of an unknown 4.3 nm phase in human SC that has a slightly weaker intensity in the vicinity of the scattering peak derived from the main short lamellar structure. Of note, Phase B is present in the human SC, which is presumed to be consistent with lattice defects. The lamellar periodicity of Phase B is shorter than that of Phase A. Given the shorter lamellar periodicity of Phase B, some possible explanations have been proposed in previous reports. Since Phase B has a crystal structure, it is unlikely that its lamellar periodicity is shortened by increasing the fluidity of the alkyl chains. To increase fluidity, the CER may adopt either a partially open alkyl chain form called a V-shaped structure or an inclined alkyl chain form. The expected length of the lamellar period for a V-shaped structure is approximately 4.2 nm, which is consistent with our results.

An increase in electron density between 0–0.5 nm in the hydrophobic region of Models 1–3 is not supported by the DECON profile in Fig. 6(a). The CER conformation of Phase B has been proposed to form a V-shaped structure in each Model, but since an increase in electron density was not observed in the hydrophobic region, we concluded that a distinct intercalation did not form.

From these results, we concluded that the conformation of CER in Phase B observed in this study was a V-shaped structure. The lipid arrangement of Phase A and Phase B is shown in Fig. 6(b). The lateral packing structure of Phase B indicates that CER[ADS] formed a V-shaped structure, neither Orth nor Hex.

In Model 3 (Fig. 3(e)), a phase transition of Phase B to the liquid crystal phase is observed at around 50 °C, whereas in Models 1 and 2, no such phase transition is observed (Figs. 3(a) and (c)). Models 1 to 3 have different lipid compositions in Phase B. Although the constituent lipids of Phase B are thought to be mostly CER and CHOL, the CHOL ratio is
higher in Phase B of Model 3 as the phase transition to lamellar liquid crystals occurs at around 50 °C. On the other hand, Phase B in Models 1 and 2 is thought to be composed mainly of CER and CHOL, but has a characteristics low CHOL ratio and characteristically high CER ratio. Moreover, Phase B in Models 1 and 2 does not undergo a phase transition to lamellar liquid crystals.

To investigate the relationship between the skin’s barrier function and short FFA species, we used PA in our study. The presence of a small amount of FFA with a short chain length of either C_{16}, C_{18}, or C_{20} in the intercellular lipid model (CER[NS] or CER_{mix}/CHOL/FFAs: 1/1/1, mol/mol) decreases the conformational order of the intercellular lipid model and increases its permeability. 45)

PA can weaken intercellular interactions in the non-polar region depending on its abundance. 12) With regards to the packing structure, studies focused on the phase transition temperature and order parameter have found that CER species containing non-hydroxy fatty acids have a denser structure than those with an α-hydroxy group. CER[AS] having an α-hydroxy group has a positive correlation with TEWL. 12) The V-shaped structure was verified in this study which formed in the intercellular lipid model using a combination of CER[ADS] with an α-hydroxy group and PA as a short-chain FFA. The V-shaped structure may occur commonly in the CER subclasses containing an α-hydroxy group.

The abundance of short FFAs is increased in damaged skin, and in the skin of patients with atopic dermatitis, which have a shorter lamellar structure than healthy skin. 4) Hereafter, it is necessary to verify whether the V-shaped structure is formed by the combination of CER containing α-hydroxy FFA subclasses and FFA of short chain length in actual skin.

In order to maintain barrier function, it is important to understand differences in the lateral packing of intercellular lipids. In this study, we proposed that the combination of CER[ADS] and PA as a short-chain FFA species, could cause the CER conformation to adopt a V-shaped structure. This structure is neither Orth nor Hex, but another crystal structure, possibly a monoclinic crystal. The lamellar structure with a lamellar period of about 4.2 nm, which was conventionally considered as an unknown phase, is considered to be a phase formed mainly of CER and CHOL, in which a CER forms a V-shaped structure similar to Phase B.

We found that temperature scanning simultaneous SAXS and WAXD measurement is a powerful tool for studying the difference in the characteristics of the packing structures. Using this method, we have elucidated the different lamellar periods between Phases A and B for the first time.

Further experiments using membrane transport experiments and temperature scanning FT-IR measurements will provide a well-defined packing structure for Phase B and clarify its relationship with barrier function. In the future, we will consider how the composition of CER subclasses and shorter fatty acid chains in the packing structure reduce barrier function. In addition, a comprehensive understanding that includes CER biosynthetic pathways is required to clarify the effect of different CER subclasses on the barrier function of intercellular lipids. We intend to pursue such studies and propose measures to control CER conformations in order to learn how the barrier function of intercellular lipids can be enhanced.

Conclusion

In the intercellular lipid model using short chain FFA and CER[ADS], two kinds of lamellar structures with different lamellar periods, Phase A and Phase B, were formed. Hydrocarbon chain packing of Phase B formed neither Orth nor Hex regardless of the lipid composition. Our results also suggest that Phase B had a V-shaped CER conformation which formed a crystal structure. This means that an increased ratio of short chain FFA in FFA species led to the malformation of a lamellar structure with a low Orth ratio that would reduce barrier function. These differences in lamellar periods and hydrocarbon chain packing are thought to be caused by differences in CER conformations. Barrier function may be enhanced by controlling the ratio of CER subclasses and the conformation of CER. The combination of FFA chain length and CER subclasses are the key.

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Conflict of Interest

The authors declare no conflict of interest.

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