

HYDROPHOBIC AND HYDROPHILIC DOMAINS IN OUTERMOST LAYER OF SKIN THAT PLAY IMPORTANT ROLE IN BARRIER FUNCTION

The outermost layer of skin, stratum corneum (SC), is composed of corneocytes and an intercellular lipid matrix (see Fig. 1). The matrix acts as both the main barrier and as a pathway for substances such as water and drugs to across SC. In mammalian SC, the longitudinal arrangement of lipid molecules, consisting of long and short lamellar structures with repeat distances of about 13.6 nm and 6 nm, respectively, has been observed by small-angle X-ray diffraction (SAXD) analysis (see Fig. 1). On the other hand, in the lateral arrangement of the lipid molecules, hexagonal and orthorhombic hydrocarbon-chain packings have been observed by wide-angle X-ray diffraction (WAXD) analysis (see Fig. 1). However, the correlation between lamellar structure (long or

short) and lateral arrangement (hexagonal or orthorhombic packing) has been unknown. On the basis of results of simultaneous SAXD and WAXD analyses of SC [1], we are now able to show that the long lamellar structure is formed by low-temperature hexagonal hydrocarbon-chain packing and that the short lamellar structure is formed by orthorhombic hydrocarbon-chain packing, as shown in Fig. 1.

There were two problems encountered when drawing the above conclusion:

- 1) At room temperature, in various mammalian SCs there are two states: (i) hexagonal hydrocarbon-chain packing with a lattice constant of 0.42 nm, (ii) orthorhombic hydrocarbon-chain packing with lattice constants of 0.42 nm and 0.37 nm. Because a lattice constant of 0.42 nm is coincidentally common to hexagonal and orthorhombic hydrocarbon-chain packings, the superimposition of the diffraction peaks of WAXD makes detailed data analysis difficult. At present, we only have evidence that when the temperature of SC rises, the orthorhombic state transforms to the high-temperature hexagonal state at 39°C; however, the transition temperature from the low-temperature hexagonal state to a high-temperature state is unknown.
- 2) Generally, one lamellar structure must be composed of one hydrocarbon chain packing. Thus, it is necessary to clarify whether the lateral packing of the lipid molecules in the long lamellar structure is low-temperature hexagonal or orthorhombic.

To solve the above problems we determined the temperature dependence of SAXD and WAXD in SC [1]. The experiments were carried out at beamline **BL40B2**. All the experiments were performed in a heating scan at a rate of 0.5 K min⁻¹. To elucidate the correlation between lamellar structure and lateral packing, analysis of hairless mouse SCs was performed with special attention given to the structural changes at phase transitions. Data from the SAXD and WAXD analyses of the SCs are plotted against temperature in Figs. 2(a) and 2(b). From results of highly sensitive differential scanning calorimetry (DSC) [2], the transition temperatures of the intercellular lipid matrix in the hairless mouse SCs were 32°C, 39°C, 51°C, 56°C, and 71°C, which are indicated by horizontal lines in Figs. 2(a) and 2(b).

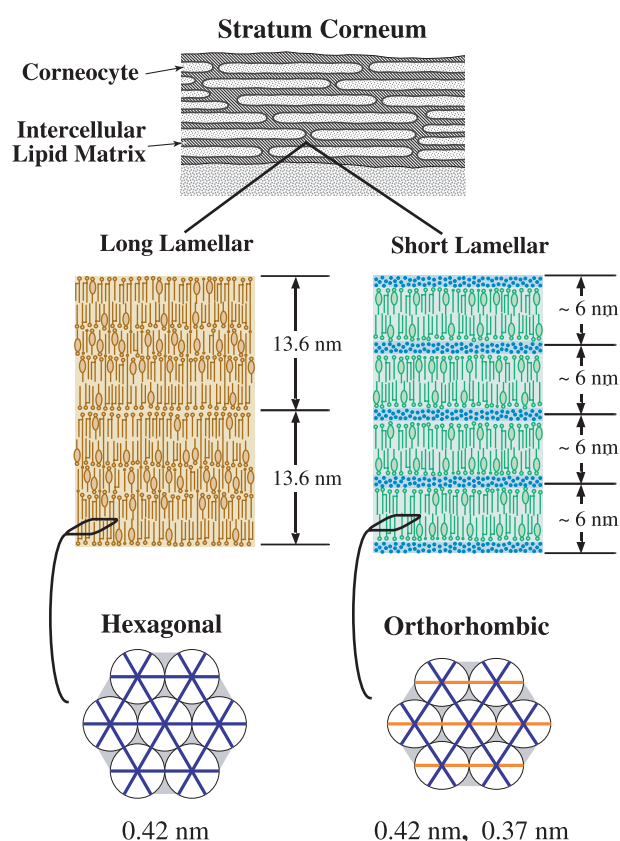


Fig. 1. The top illustration indicates a schematic model of the stratum corneum. The middle illustrations indicate molecular arrangements in the long and short lamellar structures composed of ceramides, fatty acids, cholesterol and water (blue dots represent water molecules). The bottom illustrations indicate the corresponding hydrocarbon-chain packings, that is, the low-temperature hexagonal and orthorhombic hydrocarbon-chain packings.

As seen in Fig. 2(b) below 20°C, a narrow ridge at 0.446 nm ($S = 2.24 \text{ nm}^{-1}$) appears in the intensity contour; near 32°C, it becomes a broad ridge spread over $S = 2.15\text{-}2.34 \text{ nm}^{-1}$ (note the kinks that appear in the intensity contours). From results of DSC [2], it we found that at 32°C, a phase transition from the low-temperature hexagonal state to the liquid-crystalline state takes place. On the basis of the present results, we propose that (i) the narrow ridge below 32°C, which is a precursor of the broad liquid-crystalline-like ridge, is due to cluster formation in the low-temperature hexagonal state and that (ii) at 32°C, the

low-temperature hexagonal state completely transforms into the liquid-crystalline-like state and finally becomes a broad peak near 0.46 nm ($S = 2.18 \text{ nm}^{-1}$) for the liquid-crystalline phase near 71°C (see the thick and dotted curve C in Fig. 2(b)).

By comparing the results obtained from simultaneous SAXD and WAXD analyses (in Figs. 2(a) and 2(b)), both the thick curve B, which is a trace of the diffraction peak for the short lamellar structure, and the thick curve D, which is a trace of the diffraction peak for high-temperature hexagonal hydrocarbon-chain packing resulting from orthorhombic hydrocarbon-chain packing, occur in the same temperature range from 51°C to 71°C. On the other hand, the thick curve A, which is a trace of the diffraction peak for the long lamellar structure, corresponds to the thick and dotted curve C, which is a trace of the diffraction peak for liquid-crystalline-like hydrocarbon-chain packing resulting from low-temperature hexagonal hydrocarbon-chain packing, because both curves appear in the same temperature range from 32°C to 56°C. Therefore, we propose two domains in SC, one of which is composed of long lamellar structure, which are hydrophobic in nature [3], with low-temperature hexagonal hydrocarbon-chain packing, and the other is composed of short lamellar structure, which are hydrophilic in nature [3], with orthorhombic hydrocarbon-chain packing.

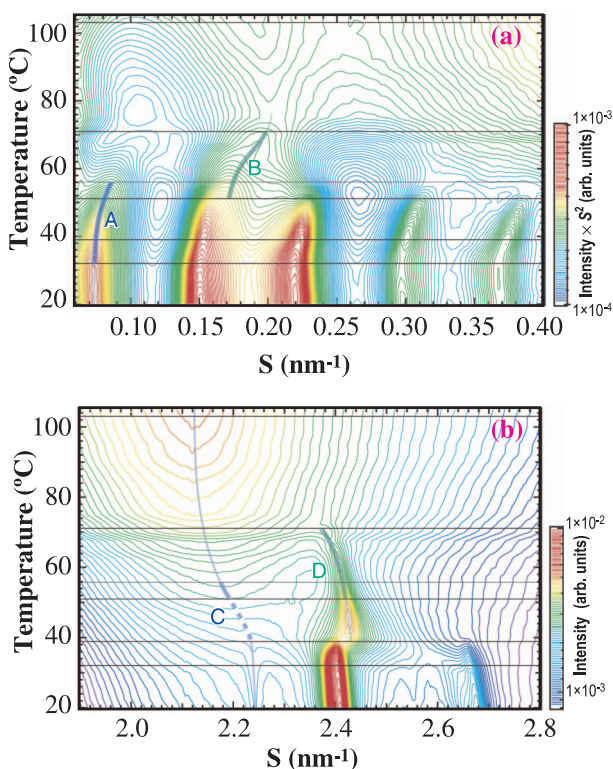


Fig. 2. Results of simultaneous SAXD and WAXD analyses of hairless mouse SCs as a function of temperature. (a) Intensity contour of SAXD analysis. (b) The intensity contour of WAXD analysis. In (a) and (b), the reciprocal spacing is expressed by $S = (2/\lambda)\sin\theta$, where λ is the wavelength of the X-ray beam and 2θ is the scattering angle. High-to-low intensity is shown by a red-to-violet scale.

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References

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