Structural Analysis of Cell Membrane Complex of Hair Fiber by Micro X-ray Beam

A mammalian hair fiber consists of the medulla, cortex and cuticle from the center to the outside in this order [1]. The major mass of hair fiber is located in the cortical region, which mainly consists of keratin filaments and matrix proteins. On the outside of the cortical region, there is cuticle which has about 5 μm in thickness and consists of about 10 keratinized sheet-like-cells. There are pathways for water molecules through the cuticle into the central cortex, one of which is the cell membrane complex (CMC) in the cuticle (Fig. 1). Recently, on the cuticle surface, covalently bound lipids mainly consisting of 18-methyleicosanoic acid have been found, which contribute to the hair surface property. Therefore, it is important to obtain detailed structures of the cortex and cuticle including water molecules to understand the penetration of water. However, since the cuticle is too thin and scatters X-rays only weakly, small-angle X-ray diffraction (SAXD) had been less applicable to the cuticle than the cortex. On the basis of a pioneering SAXD experiment using a micro X-ray beam [2], we have recently developed a SAXD technique to characterize CMC and cortex structures in a mammalian hair [3].

We performed SAXD experiments using a micro-beam at beamline **BL40XU**. A high-flux X-ray beam (λ = 0.083 nm, wavelength) from a helical undulator was focused with two mirrors. In the experimental hutch, an X-ray beam of 5 μm in diameter was obtained behind two pinholes, the first 5 μm and the second 100 μm in diameter. The sample-to-detector distance was about 2.4 m. The reciprocal spacing S (= $1/d = (2/\lambda)\sin(2\theta/2)$, 2θ is the scattering angle and d is the repeat distance) was calibrated by the spacing of standard materials at room temperature.

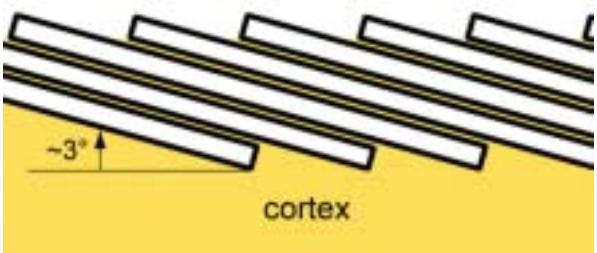

Fig. 1. Schematic cross section of mammalian hair cuticle on cortex.

From the SAXD profile of CMC (Fig. 2(a)), the equator of CMC is tilted by about 3º with respect to the equatorial direction of the fiber axis, in accordance with the structural evidence from an electron microscopic observation. Figure 2(b) is similar to the SAXD profile of the bundle of mammalian fibers. Thus, the present experiment provides structural information on CMC without interference from other structures.

CMC in the cuticle of a mammalian hair fiber is composed of three layers, which are the β, δ and β layers. It has been assumed that the electron density profile of CMC can be expressed by box-type wells. The δ and β layers are composed of proteins with flexible shapes and lipid molecules of various hydrocarbon-chain lengths, respectively. When we take into account the variation of thickness in the δ and β layers, overall features of the SAXD profile (Fig. 3) can be explained satisfactorily [3].

We focused our attention on an influence of humidity on the cortex and cuticle structures in a rat whisker. A rat whisker sample was incubated at a relative humidity (RH) between 3% and 100% which was controlled by a humidity generator (Model HUM-1, Rigaku).

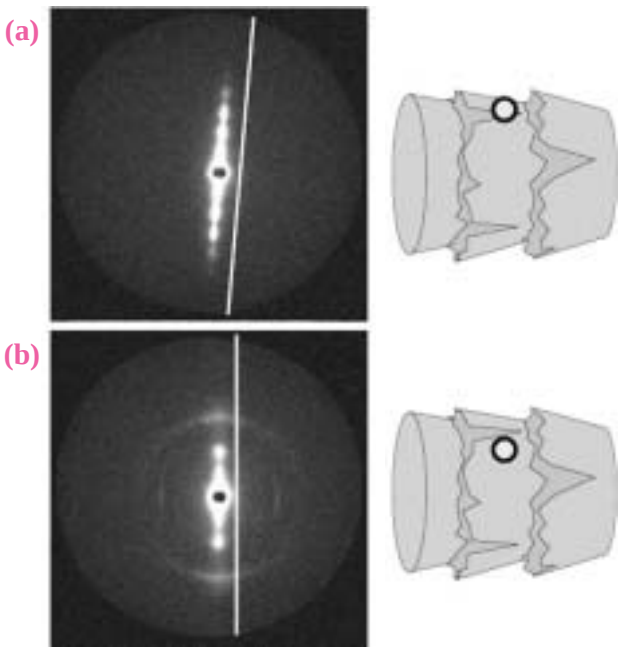

Fig. 2. SAXD patterns (left), and schematic drawing (right) of rat whisker using micro-beam X-ray, which is denoted by a circle. **(a)** cuticle, **(b)** cortex.

Whisker diameter was estimated from the distance between points on two sides of a rat whisker where the maximum intensity from the cuticle was observed. The diameter normalized by that at 3% RH is shown in Fig. 4. In addition, the first peak position was obtained in the cortex region from SAXD patterns using a micro X-ray beam. Similarly, the peak positions were normalized by that at 3% RH, which are shown in Fig. 4. It is clear that the normalized diameter corresponds to the normalized peak position over a wide humidity range. Thus, the whisker diameter is mainly characterized by the cortex structure.

CMC thickness was estimated on the basis of a CMC structural model to fit X-ray diffraction profiles of cuticle. The normalized thickness is shown in Fig. 4. As the present results indicate, the swelling property clearly differs between normalized CMC thickness and whisker diameter. CMC thickness gradually increased below 80% RH, whereas it markedly increased over the normalized whisker diameter above 80% RH. These results suggest that free water molecules penetrate into the cortex and cuticle in different manners.

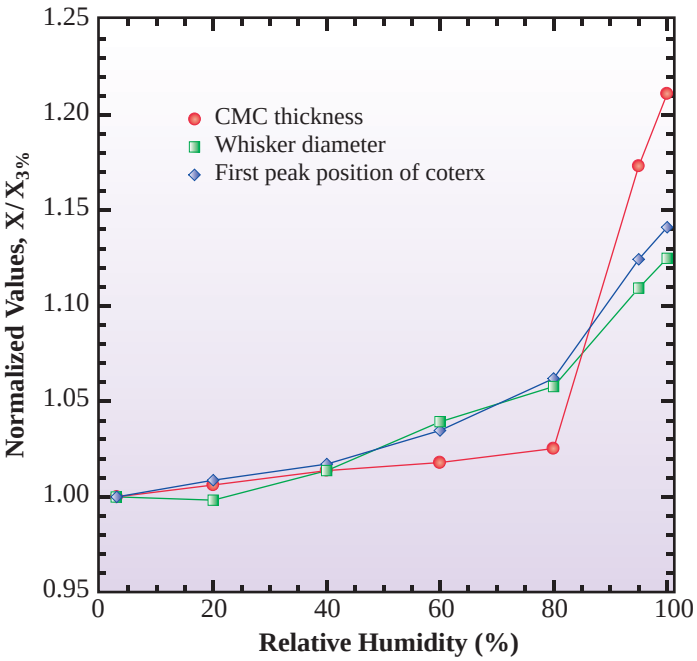

Fig. 4. CMC thickness (circle), whisker diameter (square) and first peak position of cortex (diamond), which are normalized by the value at 3% RH.

Thus, in SAXD studies using a micro-beam on the pathways of water, a rat whisker will be a promising sample for a detailed structure analysis. For cosmetic applications, we believe that our method will be useful for studying the influence of shampoo, rinse or permanent solution on the thickness of CMC, and the β and δ layers of the mammalian hair cuticle.

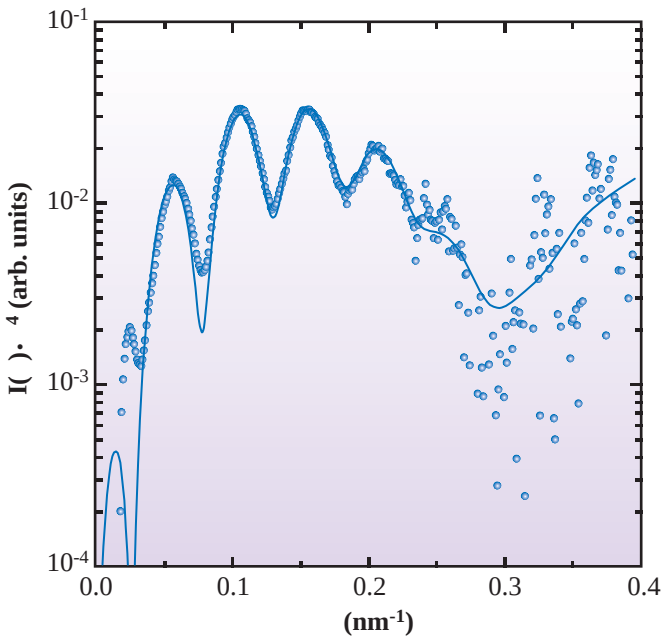

Fig. 3. SAXD profile of rat whisker and fitting result.

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References

[1] C.R. Robbins: Chemical and Physical Behavior of Human Hair, 4th ed., (2001) New York, Springer.
[2] L. Kreplak *et al.*: Biochim. Biophys. Acta **1547** (2001) 268.
[3] *N. Ohta, T. Oka, K. Inoue, N. Yagi, S. Kato and I. Hatta: J. Appl. Cryst.* ***38*** *(2005) 274.*