J. Cosmet. Sci., **58**, 11–17 (January/February 2007)

Structural analysis of the cell membrane complex in the human hair cuticle using microbeam X-ray diffraction: Relationship with the effects of hair dyeing

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Accepted for publication September 20, 2006.

Synopsis

This article deals with the structure of the cell membrane complex (CMC) in the human hair cuticle. The microbeam X-ray provided a pattern of small-angle scattering from the CMC in the cuticle with no sample preparations, including slicing and pre-staining of hair. The thickness of the β- and δ-layers, substructure in CMC, was estimated by analysis of the scattering pattern. We used hair samples extracted with several solvents, and found that solvent extraction changed the thickness of the β- and δ-layers in a manner dependent on the type of solvent. Extraction of hair with solvent was also shown to have effects on the extent of dyeing. There was a high correlation between the extent of dyeing and the thickness of the δ-layer, i.e., a thin layer tended to show a high amount of dyeing, whereas there was no significant correlation between the thickness of the β-layer and the extent of dyeing.

INTRODUCTION

The cuticle is the outermost layer of a hair fiber and is made up of a stack of approximately ten sheet-like cells that is 0.5 μm thick and roughly 60 μm square. The surface of each cell is covered by a thin layer of lipids called the β-layer (2.5 to 4.0 mm thick), and these lipids are separated between adjacent cells by the δ-layer (15 to 18 nm thick), which acts as an intercellular cement (1). The exact composition of the δ-layer is still unknown; however, it has been proposed to contain non-keratinous proteins (2). This lipid–protein cement–lipid structure is called the cell membrane complex (CMC) and is the only substructure that continuously fills the intercellular spaces of hair fibers.

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Therefore, it is considered to be an important pathway for the penetration of substances into the inner parts of the hair (3). Observations of cuticles by transmission electron microscopy have shown evidence of a penetration pathway through the CMC (4,5); however, that observation method requires the use of heavy metals and thin-sliced hair samples. We considered that a less invasive technique is needed to understand the penetration properties of the cuticle.

In the present study, we utilized microbeam X-ray diffraction for structural analysis of the CMC in the hair cuticle. With this method, we were able to elucidate the structure of the CMC in whole hair samples under ambient conditions without pretreatment, such as staining with heavy metals. The pioneering experiment on the structure of the cuticle using a microbeam X-ray technique was performed by Kreplak *et al.* (6), in which they observed small-angle X-ray scattering (SAXS) patterns of cuticles, and determined the average thickness values of the β- and δ-layers in the CMC. Thereafter, a mathematical model for precise estimation of the thickness of those layers was proposed by Ohta *et al.* (7). Using that model, we determined the thickness of the β- and δ-layers in hair fibers treated with various solvents. Our results showed a relationship between the CMC structure and the extent of hair dyeing as a possible indicator of penetration.

MATERIALS AND METHODS

HAIR SAMPLES

For SAXS-pattern experiments, hair strands were obtained from Japanese women who had not used any chemical treatments and were then washed with a 0.2% sodium laureth sulfate solution. After being rinsed with deionized water and drying on a paper towel, the strands were subjected to extraction with four different solvents, methanol, acetone, hexane, and a chloroform/methanol mixture (2:1 v/v) at 37°C for six hours. After cutting to a length of 8 cm, each hair fiber was fixed on a hair holder and subjected to SAXS analysis.

DYEING EXPERIMENTS

For the dyeing experiments, strands of gray hair (1 gram, 10 cm; BM-W, Beaulax, Tokyo, Japan) were used and subjected to extraction with the four solvents, using the same method noted above. The hair tresses were then soaked in a solution containing 3.0 g of acid orange 7, 9.0 g of citric acid, and 1.0 g of sodium citrate per liter (pH 2.7) at 35°C for five minutes. After rinsing the tresses with deionized water and drying, the color index [light (L), red (a), and yellow-blue (b)] of each hair strand was determined using a chromometer (CR-400, Konica Minolta Sensing, Osaka, Japan). The extent of dyeing was then determined by calculating the differences in the indexes between untreated and dyed hair samples (i.e., ΔL, Δa, Δb) using the following formula: dyeing extent (ΔE) = SQRT{$(\Delta L)^2$ + $(\Delta a)^2$ + $(\Delta b)^2$}.

SAXS EXPERIMENT

SAXS experiments were performed with a 5-μm high-flux beam (BL40XU High Flux Beamline) at the SPring-8 facility (Hyogo, Japan) using human hair fibers under an

atmosphere of 30°C and 50% relative humidity. A high-flux beam emitted from a helical undulator (λ = 0.083 nm) was focused with two mirrors laid horizontally and vertically (8). In an experimental hutch, an X-ray beam 5 μm in diameter was produced behind two pinholes, the first 5 μm in diameter and the second 100 μm in diameter. The sample-to-detector distance was set at approximately 2.3 m. The reciprocal spacing (S) was calibrated by a spacing of 4.894 nm for lead stearate. The X-ray diffraction profile was recorded by a two-dimensional detector with an exposure time of 1 second, using an X-ray image intensifier coupled to a cooled CCD camera (1024 × 1024 pixels). The thickness of the β-layer and δ-layer was estimated using the method of Ohta *et al.* (7).

RESULTS

EFFECTS OF SOLVENT EXTRACTION ON HAIR DYEING

The effects of solvent extraction on the properties of hair dyeing are shown in Figure 1. All of the solvents used in the present study elevated the extent of dyeing as compared with that of non-extracted hair fibers. The differences seemed slight but have statistical significance. There were differences in the extent of hair dyeing among the solvents used, as hair subjected to extraction with hexane tended to become smaller.

DIFFRACTION FROM CUTICLE

An illustration of a SAXS diffraction pattern from a portion of the cuticle is shown in Figure 2, in which signals vertical to the hair axis are tilted approximately 3° with an

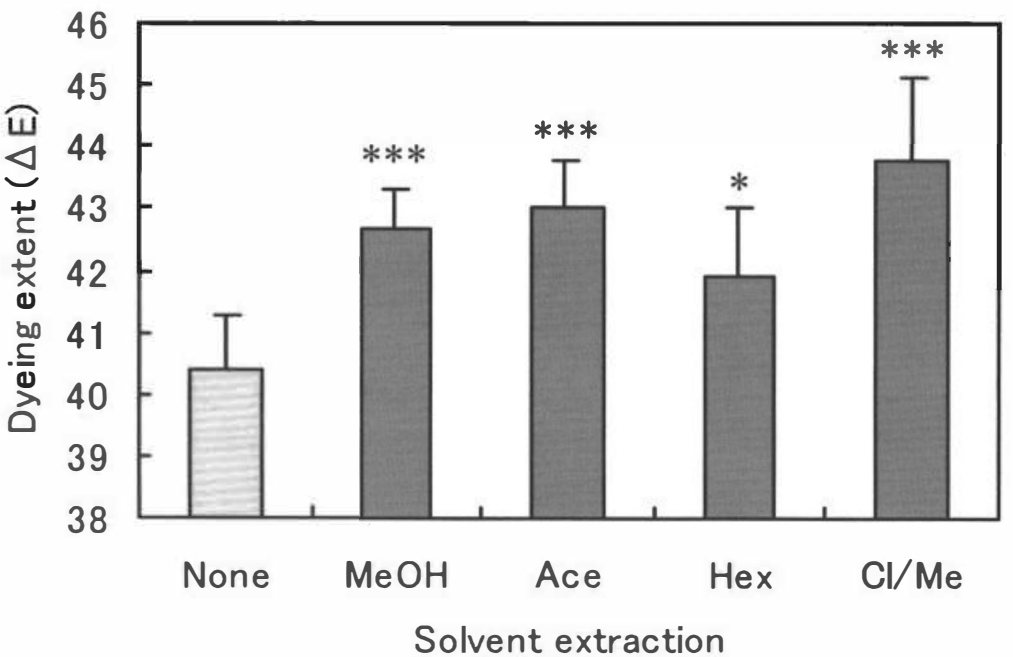

Figure 1. Effect of solvent extraction on hair dyeing properties. None: non-extracted. MeOH: extracted with methanol. Ace: extracted with acetone. Hex: extracted with hexane. Cl/Me: extracted with a mixture of chloroform and methanol (2:1). Mean ± standard derivation (n = 8). Statistical significance was analyzed using a Dunnett test. $*p < 0.05$, $***p < 0.001$.

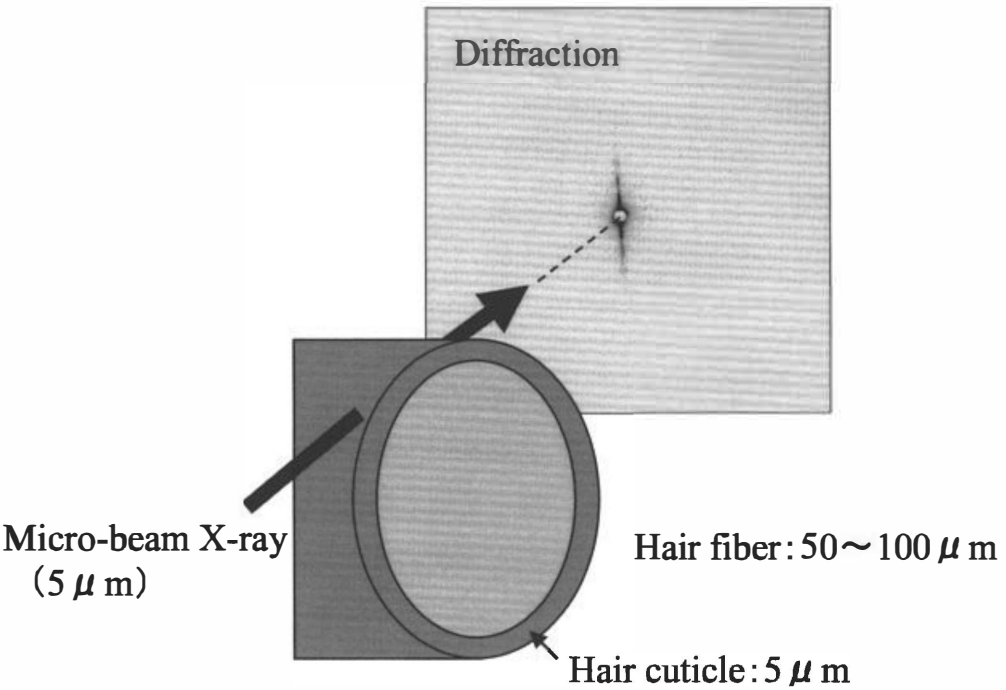

Figure 2. SAXS experiment.

equal tilt to the hair axis. This diffraction pattern shows the same profile that was estimated to diffract from the cuticle CMC in previous reports (6,7).

A one-dimensional intensity profile produced from the diffraction pattern is demonstrated in Figure 3. The intensity [$I(S) \times S^4$] was plotted versus reciprocal spacing (S) according to an analytical method proposed by Ohta *et al.* (7). Reciprocal spacing (S) was used to represent the index for the distance from the center of the diffraction pattern, where $S = 1/d = (2/\lambda) \times \sin(2\theta/2)$, with λ representing the wavelength of the X-ray, 2θ the scattering angle, and d the repeat distance. Using this method, we estimated the thickness of the β- and δ-layers in the CMC. The region of $S < 0.2$ shown in Figure 3 contains four peaks of $I(S) \times S^4$. The spans of the peaks differed among the hair samples, which reflected differences in CMC structure. All intensity profiles analyzed in the present study contained at least three peaks of $I(S) \times S^4$ in the region of $S < 0.2$. The analytical region ($S < 0.2$) was considered wide enough for correct estimation of the thickness of the β- and δ-layers.

EFFECTS OF SOLVENT EXTRACTION ON CMC STRUCTURE

SAXS experiments were performed using hair samples extracted with the four solvents. The estimated values of thickness of the β- and δ-layers differed, based on the solvent used, as shown in Figure 4. The thickness of the β-layer was decreased by extraction with acetone and hexane, while it was not changed by extraction with methanol or chloroform/methanol. In addition, the thickness of the δ-layer was decreased by extraction

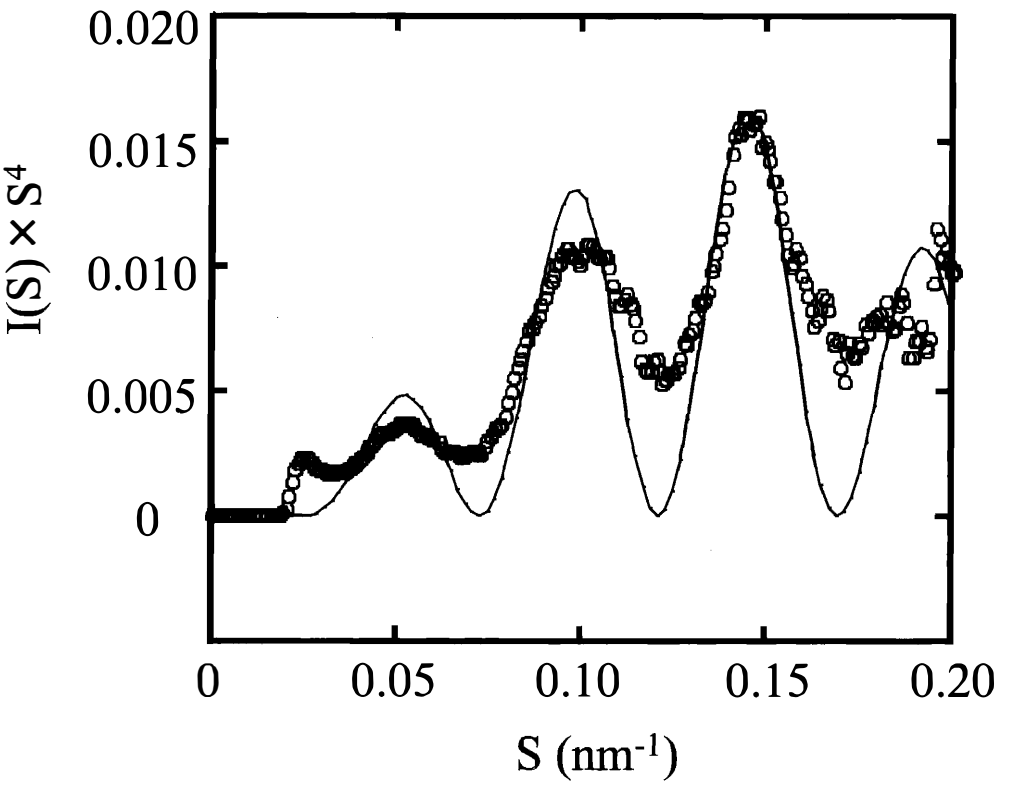

Figure 3. One-dimensional equatorial profile extracted from SAXS pattern, obtained as shown in Figure 2. We estimated the thickness of the β- and δ-layers from the one-dimensional equatorial profile according to the method of Ohta *et al.* (7), using the following formula: $I(S) \times S^4 = a \times \{\cos[\pi \times S \times (\beta + \delta)]\}^2 \times \{\sin(\pi \times S \times \beta)\}^2$, where S is reciprocal spacing [$=(2/\lambda)\sin(2\theta/2)$] and I is intensity.

with methanol, acetone, and chloroform/methanol, and did not significantly change with hexane.

The relationships between the CMC structure and extent of hair dyeing are shown in Figure 5. The average values for the extent of hair dyeing (ΔE) with each of the treatments shown in Figure 1 were plotted with the average values of thickness of the β- and δ-layers shown in Figure 4. There was a significant correlation between the extent of hair dyeing and the thickness of the δ-layer, whereas no correlation was seen between the extent of hair dyeing and the thickness of the β-layer.

DISCUSSION

Using a microbeam X-ray method, we observed the CMC structure without the prestaining or slicing of hair fibers under typical ambient conditions (30°C, 50% relative humidity). We considered that the present SAXS technique was able to obtain information regarding hair structure under natural conditions, as the hair samples did not require prestaining. Structural changes in the cuticle CMC were found to be caused by extraction with the various solvents, which seemed to reflect their hydrophobicity. Methanol, which has

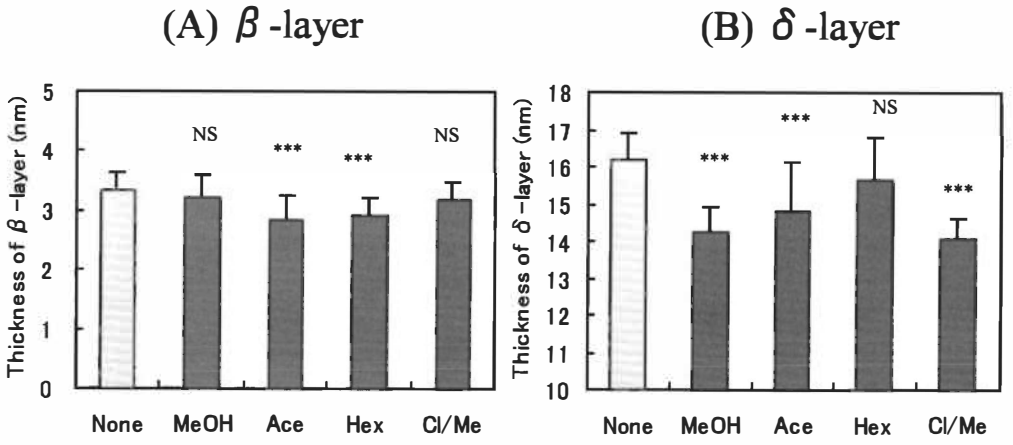

Figure 4. Effects of extraction with solvents on the thickness of the β- and δ-layers. (A) β-layer. (B) δ-layer. None: non-extracted. MeOH: extracted with methanol. Ace: extracted with acetone. Hex: extracted with hexane. Cl/Me: extracted with a mixture of chloroform and methanol (2:1). Mean ± standard derivation (four hair lots, each n = 7). Statistical significance was analyzed using a Dannett test. $^{NS}p > 0.05$, $^{***}p < 0.001$.

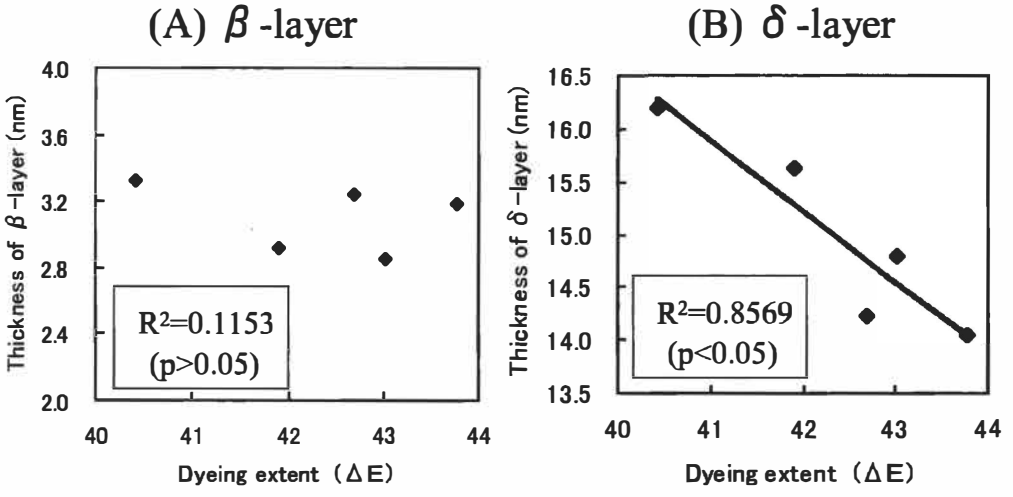

Figure 5. Relationships between the CMC structure and the extent of dyeing. (A) Relationship between the thickness of the β-layer and the extent of dyeing. (B) Relationship between the thickness of the δ-layer and the extent of dyeing.

hydrophilic characteristics, demonstrated a strong effect on the δ-layer (protein layer), whereas hexane, which has hydrophobic characteristics, primarily had an effect on the β-layer (lipid layer). In addition, acetone, with an intermediate hydrophobicity between methanol and hexane, had effects on both the δ- and β-layers, while the effect of the mixture of chloroform/methanol was similar to that of methanol.

Our results showed that extraction with solvent of elevated the extent of dyeing in human hair. It was previously reported that solvent extraction accelerated the dyeing rate of wool fiber (9). The differences in the extent of dyeing seen in the present study with the different solvents were apparently related to the changes in the dyeing rate rather than changes in dye-binding capacity. The dyeing period used (five minutes) was rela-

tively short, and our preliminary experiment shows that dye binding for that amount of time was approximately 25% to saturated level.

Our findings showed a correlation between the extent of dyeing and the thickness of the δ-layer, which was changed by extraction with the solvents, with a larger decrease in thickness resulting in a greater elevation in the extent of dyeing. It has been speculated that hydrophilic molecules penetrate hair through the δ-layer, based on histochemical observations of the CMC (10). Since the dye used in our study (acid orange 7) was water-soluble, the relationship seen between the extent of dyeing and δ-layer thickness is in agreement with that proposal. Thus, using a microbeam SAXS method, we were able to detect changes in the CMC structure that correlated with the penetration of molecules.

CONCLUSION

Microbeam SAXS is a useful tool for hair and cosmetic science. This provides structural information regarding the cuticular CMC, without the pre-staining or slicing of hair samples. Using microbeam SAXS, we found CMC structural changes caused by solvent extraction correlating with changes in the penetration of molecules into the hair. Thus, using a microbeam SAXS method, we were able to detect changes in the CMC structure that correlated with the penetration of molecules.

ACKNOWLEDGMENTS

The synchrotron radiation experiments were performed at the SPring-8 facility with the approval of the Japan Synchrotron Radiation Research Institute (JASRI) (Proposal No. 2004B0485-NL2-np).

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