Structural Analysis of the Outermost Hair Surface Using TOF-SIMS with C$_{60}$ Depth Profiling Technique*

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Components on the outermost hair surface were directly analyzed with time-of-flight secondary ion mass spectrometry (TOF-SIMS). The depth profiling of characteristic amino acid components in hair surfaces was performed using a Bi$^{++}$ analysis beam combined with a C$_{60}^{++}$ sputtering beam. The depth analysis of a protein bilayer model of zein and casein layers indicated that the damage caused by C$_{60}^{++}$ sputtering did not influence the TOF-SIMS analysis, therefore, the C$_{60}$ sputtering is considered to be feasible at an analysis depth of 2 $\mu$m from the surface. The depth profiling analysis data for hair surfaces showed oscillating changes in the peak intensities of amino acid fragments, and this phenomenon is considered to reflect the multilayer structure of cuticle tissue. Focusing on the first layer of cuticle tissue, the depth profiling change of the cysteine/cystine-related fragment corresponding to the A-layer, exocuticle and endocuticle of cuticle tissue was consistent with the TEM result. Furthermore, a comparison of the depth profiling data from before and after the bleaching treatment revealed that the cysteine/cystine-related peak intensity was high at the outermost surface because the layer was cysteine-rich for bonding the lipid layer. As a result, the existence of a thin protein layer with specific amino acid components, known as the epicuticle, was suggested. The depth profiling analysis of protein using TOF-SIMS provides significant information for investigating the structure and functions of the surface of human hair.

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I. INTRODUCTION

The structure of human hair comprises highly organized strata covered with multiple layers of cuticle cells. The cuticle cell proteins have a high cross-link density [1] and protect the inner cortical tissues of human hair. The surface properties of the cuticle play important roles in hair’s smoothness to the touch and in suppressing hair tangles [2]. Thus the morphology and chemical structures of cuticle cells provide significant information for investigating the structures and functions of human hair. It is well known that the outermost surface of the cuticle is covered with a monolayer of covalently bonding lipids, mainly 18-methyleicosanoic acid (18-MEA) [3], and that the tissue layers, A-layer, exocuticle and endocuticle [4,5], are under the lipid layer. Furthermore, there is another tissue layer with different amino acid composition, called the epicuticle [6], between the lipid layer and A-layer. The studies of the epicuticle have been performed using component analyses [7] or microscopic observations [8]. However, the physical properties and functions of the epicuticle have not been clarified sufficiently, because the epicuticle is so thin [8] that it is difficult to analyze separately from other tissues. In this study, the components of the hair surface were directly analyzed with time-of-flight secondary ion mass spectrometry (TOF-SIMS).

TOF-SIMS is a powerful tool for the analysis of solid surfaces and the imaging analysis of organic molecules with submicron resolution. Since 2003, cluster ion sources such as C$_{60}$ [9] and large argon cluster ions [10] have been used in TOF-SIMS analysis. The main features of these ion sources are a high secondary ion yield for organic molecules and low damage sputtering of organic mate-

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rials [9-12]. In particular, the low damage sputtering enables the molecular depth profiling of organic materials. For example, the analysis of polymers [13,14] and biological cells [15] and analysis using an organic photoluminescence device [16] have been reported. In this study, human hair surface structures were analyzed using the TOF-SIMS depth profiling technique with C60 sputtering.

Natural surface lipids, silicone and surfactants adsorbed on the hair surface are detected with TOF-SIMS. Furthermore, fragments of amino acid residues are detected in a low mass range [17], and the amino acid compositions of proteins can be analyzed by monitoring these peaks. The depth profiling of amino acid components can be performed using a Bi3++ analysis beam combined with a C60 sputtering beam. In this study, the depth profiling of a protein bilayer model of zein and casein layers was performed in order to confirm the feasibility of protein analysis using C60 sputtering. After that, the human hair surface structure was examined using TOF-SIMS depth profiling and comparing these results to transmission electron microscopy (TEM) results.

II. EXPERIMENTAL

A. Sample preparation

The hair samples were prepared using chemically untreated hair fibers provided by a Japanese female, age 30. Segments 5 cm from the root of the hair fibers were employed in this analysis, and the fibers were washed by soaking in an organic solvent (chloroform-methanol-water 18:9:1 (v/v/v)) for 24 hours prior to the measurements. The cleaned hair sample was prepared by exposing the sample fibers to conventional bleaching lotion (Table I) at the same liquor-to-fiber ratio for 20 minutes at room temperature. After bleaching, the hair was rinsed for 1 minute under running water.

The depth profiling of the protein bilayer film of zein and casein was conducted to investigate the feasibility of the C60 sputtering. For the bilayer film preparation, a casein solution was spin-cast directly onto the first layer to form the upper layer of zein. Water 9:1 (v/v) solution containing zein was spin-cast directly onto the substrate of the silicon wafer to form the under layer of casein, and a ethanol-water 9:1 (v/v) solution containing casein was spin-cast onto the substrate of the silicon wafer to form the under layer of casein. The intensity changes of these peaks were consistent with the components of zein and casein [20]. Additionally, the

B. TOF-SIMS and TEM analyses

TOF-SIMS measurements were performed with a TOF-SIMS V instrument (ION-TOF GmbH, Münster, Germany) using a focused 25 keV Bi3++ primary ion beam at a typical current of 0.3 pA. The data were collected by scanning the primary ion beam over an analysis area of 50×50 μm using high mass resolution mode (m/Δm=6000; lateral resolution, 2 μm). Charge compensation of the sample was performed by irradiating the sample with a pulsed beam of electrons using an electron flood gun.

Table II summarizes the results of the amino acid analysis of cuticle cells [1] and the fragment ions detected by TOF-SIMS for each amino acid residue [19]. In this study, the distributions of the main components of the amino acids cysteine/cystine (Cys/Cyc), serine, proline, glutamic acid and valine were investigated.

Figure 1 shows the depth profiling of the protein bilayer model. The x-axis on the graph represents the sputtering time which correspond to the analysis depth, and the y-axis represents the ion intensity for each amino acid residue. In Fig.1, the changes in peak intensity were observed after approximately 80 seconds, which corresponded to an interface region of the bilayer film. Compared to the upper layer, the underlayer was rich in valine and the glutamine and glutamic acid peaks were lower. The intensity changes of these peaks were consistent with the components of zein and casein [20]. Additionally, the

Table I. Formulation of bleach lotion

| Ingredient                                | Concentration |
|-------------------------------------------|---------------|
| 35% hydrogen peroxide                     | 3.5 wt%       |
| 28% ammonia water                         | 0.9 wt%       |
| Ammonium bicarbonate                      | 5.6 wt%       |
| Hexadecyl trimethyl ammonium chloride     | 1.25 wt%      |
| Hydroxyethylidene Diphosphonic Acid       | 0.05 wt%      |
| Water                                     | Balance       |

FIG. 1. Depth profiling of the protein bilayer model. The upper layer is zein and the underlayer is casein.
peak intensity of each secondary ion in each layer was stable, thus, the damage caused by C\textsubscript{60} sputtering was not observed during the measurement. The thickness of the upper zein layer was calculated as 700 nm, and the total sputtering depth was estimated to be nearly 2 \mu m. Therefore, C\textsubscript{60} sputtering is feasible for the depth profiling of protein components at an analysis depth of 2 \mu m from the surface.

Figure 2 shows the TEM image of the hair surface cross-section and the TOF-SIMS depth profiling results of the solvent-washed hair surface. TOF-SIMS measurements were performed over an analysis area of 50\times 50 \mu m; the data represent the average profiling of several cuticle cells. In the depth profiling data, it is characteristic that the amino acid peak intensities oscillate. The oscillating change in the peak intensities was examined from the structure of human hair surface. As shown in the TEM image, the surface of the human hair was surrounded with 5 to 10 multilayers of cuticle tissues [21], and a single layer of cuticle tissue consists of several tissues [4-6]. The oscillating change in the peak intensity is considered to reflect the multilayer structure of the cuticle tissue. The depth resolution decreased gradually because the hair surface is not flat and the thicknesses of the cuticle layers are not even. Furthermore, in the deeper area of 1 \mu m, the 3rd to 5th layers of the cuticle, the accumulation of sputtering damage also influences the decrease in the depth resolution. As a result, the depth profile of the cuticle cells under the second layers became ambiguous. In this study, the precise structural analysis was performed in the first cuticle layer.

Figure 3 shows the TEM observation of cuticle cells and the depth profiling data of Cys/Cyc in the first cuticle layer. In the detection of the Cys/Cyc peak (m/z 76), it was confirmed that the overlapping of other peaks, such as C\textsubscript{6}H\textsubscript{4}N\textsubscript{2}, was small; thus the variation of peak intensity mainly reflected the change in Cys/Cyc composition [22]. It is indicated that a single layer of cuticle consists of several

| Amino acid component analysis of cuticle | Fragment peaks detected by TOF-SIMS |
|-----------------------------------------|-----------------------------------|
| Amino acid | Ratio(%) | Chemical structure of fragment | Fragment mass number (amu.) |
| Cysteine/Cystine | 24.1% | C\textsubscript{2}H\textsubscript{6}N\textsubscript{2} (Cys/Cyc) | 76.02 |
| Serine | 16.1% | C\textsubscript{2}H\textsubscript{6}NO (Ser) | 60.05 |
| Proline | 11.4% | C\textsubscript{4}H\textsubscript{6}N (Pro) | 68.05 |
| Glutamic acid | 9.4% | C\textsubscript{4}H\textsubscript{6}NO (Glu/Glu) | 84.05 |
| Valine | 7.3% | C\textsubscript{4}H\textsubscript{8}N\textsubscript{2} (Val) | 72.09 |
| Glycine | 7.0% | CH\textsubscript{2}N (Gly) | 30.03 |
| Threonine | 6.0% | C\textsubscript{4}H\textsubscript{4}O (Thr) | 69.04 |
| Leucine | 4.8% | C\textsubscript{5}H\textsubscript{12}N (Ile/Leu) | 86.10 |
| Arginine | 4.1% | CH\textsubscript{3}N\textsubscript{2} (Arg) | 43.03 |
| Aspartic acid | 3.3% | C\textsubscript{3}H\textsubscript{5}NO\textsubscript{2} (Asn/Asp) | 88.04 |
| Isoleucine | 2.1% | C\textsubscript{2}H\textsubscript{5}N\textsubscript{2} (Ile/Leu) | 86.10 |
| Tyrosine | 1.5% | C\textsubscript{7}H\textsubscript{7}O (Tyr) | 107.05 |
| Phenylalanine | 1.0% | C\textsubscript{9}H\textsubscript{10}N (Phe) | 120.09 |
| Cysteic acid | 0.8% | – | – |
| Methionine | 0.4% | C\textsubscript{2}H\textsubscript{5}S (Met) | 61.01 |
| Alanine | – | C\textsubscript{2}H\textsubscript{6}N (Ala/Cys) | 44.05 |
| Lysine | – | C\textsubscript{6}H\textsubscript{10}N (Lys) | 84.09 |
| Histidine | – | C\textsubscript{4}H\textsubscript{2}N\textsubscript{2} (His) | 81.04 |

FIG. 2. Comparison of the TEM image of the hair surface cross-section (a) and the TOF-SIMS depth profiling data (b).

FIG. 3. TEM image of the hair surface cross-section of cuticle tissue (a, b) and TOF-SIMS depth profiling of the first layer of cuticle tissue (c).
eral tissue layers, as a consequence of the peak intensity changes resulting from the difference in the Cys/Cyc composition of each tissue. The depth profiling data mainly consist of three segments identified as the A-layer, the exocuticle and the endocuticle. In order to confirm the assignment of the layers, the TOF-SIMS data were compared to the TEM results in terms of the Cys/Cyc intensity change. In the high magnification TEM image of a single cuticle layer, the image contrast represented the difference in Cys/Cyc contents because methenamine-silver staining mainly reacts to thiol and disulfide moieties [18]. Therefore, the Cys/Cyc content is high in the A-layer and becomes lower in the exocuticle and endocuticle layers [18,23]. Thus, the depth profiling change in the Cys/Cyc component obtained by TOF-SIMS measurement was consistent with the TEM result.

The outermost surface structure consists of several tissue layers on top of the A-layer, although the fine structure was not distinguished by TEM observation. Figure 4 shows a schematic of the chemical structure of a human hair’s surface. On the outermost surface, there is a lipid layer binding to proteins mainly via thioester bonds with cysteine residues [24] as shown in Fig.4. Moreover, the existence of a protein layer of specific components, called the epicuticle, was suggested, although the evidence for the existence of the epicuticle is insufficient because the epicuticle is so thin that it is difficult to analyze separately. It is remarkable that the initial intensity in the

FIG. 4. Schematic of the chemical structure of a human hair surface.

FIG. 5. Schematic of the change in chemical structure before (a) and after (b) the bleaching treatment.

TOF-SIMS depth profiling (Fig.3) was extremely high, which suggested that there is a Cys/Cyc-rich layer on the outermost surface of human hair.

The factors influencing the initial change in the Cys/Cyc peak intensity was examined. It was reported that the lipid layer on the outermost hair surface was eliminated by a bleaching treatment [25], and therefore cysteine bonding to lipids is considered to be oxidized to cysteic acid as shown in Fig.5. Thus the Cys/Cyc peak reflecting cysteine binding to lipids was not detected after the bleaching treatment. Figure 6 shows a comparison of the depth profiling of the Cys/Cyc peak before and after the bleaching treatment. The change in the Cys/Cyc peak intensity at the outermost layer was not recognized in the bleached hair. The initial change observed in the depth profiling data was due to the cysteine residues binding to the lipid. From these results, it was concluded that there is a cysteine-rich layer at the outermost hair surface, and this layer plays an important role in the formation of the bonding lipid layer. Additionally, the existence of epicuticle tissue, which is explained as a thin surface layer with different amino acid components, was suggested by TOF-SIMS depth profiling analysis. These results provide significant information for investigating the physical properties of the surface of human hair.

IV. CONCLUSION

The surface structure of human hair was examined by TOF-SIMS depth profiling analysis of amino acid components using a Bi$_3$ analysis beam combined with a C$_60$ sputtering beam. In the comparison of the TOF-SIMS depth profiling to the TEM observation results, the oscillating changes in the amino acid components were observed, which reflects the multilayer structure of the cuticle tissue. Furthermore, TOF-SIMS depth profiling of a surface region indicates that a cysteine-rich layer exists on the outermost hair surface and plays an important role in the formation of the surface lipid layer. Thus, the TOF-SIMS depth profiling analysis revealed the existence
of a thin layer with different amino acid components, suggesting the existence of epicuticle tissue.

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