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New aspects of the structure of human hair on the basis of optical microscopic observations of disassembled hair parts

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Synopsis

Infant' and adult' scalp hair fibers were disassembled to various cellular components and blocks by chemical and enzymatic treatments, followed by random scission with rapidly rotating cutters. The hair fibers were also fractured by the use of a vise. The optical microscopic inspection of these specimens led to the discovery of many previously unknown structures in the hair shaft. In particular, a cuticular cell (Cu) was found to take a trowel-like shape consisting of a part with a blade-like shape (CuB) and a part with a handle-like shape (CuH), where CuB overlapped one another and fused partially to build the honeycomb-like structure on a large cuticular thin plate (CuP). Whereas CuH was closely similar to the cortical cell in dimensions and richness of macrofibrils (Mf). It was considered that human hair is stabilized structurally and physicochemically by the presence of the honeycomb-like structure, the CuP and the Mf.

INTRODUCTION

Several studies on the science of human hair were carried out over the last several decades, using the traditional structural model that was chiefly composed of spindle-like cortical cells (Co), flat cuticular cells (Cu), and serially aligned medulla cells (1–3). It would be very important, especially in a cosmetic field, to analyze how chemical and biochemical agents interact with these cellular components. As a matter of fact, mammals' hair fibers have been frequently investigated by electron microscopes (4–15). The technique, however, even ignoring a laborious process for the preparation of a specimen, works *in vacuo* and provides only a black-and-white photograph of the dried substance. On the other hand, optical microscopes have been conveniently employed for examination of various hair samples despite the fact that the resolution is limited to semimicro and micrometer levels. Indeed, the microscopy is well suited to dealing with wet substances, but also is very useful to inspect small objects for the structural characteristics, providing a see-through image with a high depth of field and permitting chromatic distinction between two similar matters in the specimen.

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The purpose of this study was to discover the unknown structures hidden in the inner domain of a human hair shaft. For achieving this aim, scalp hairs were disassembled or fractured to cellular components and blocks by combining chemical or enzymatic treatment with special cutting and compression processes. Taking advantages of the aforementioned optical microscopy, many specimens were analyzed for the inner structure of the hair shaft.

EXPERIMENTAL

HAIR SAMPLES AND REAGENTS

Two- and six-year-old Japanese girls kindly gave black hairs I and II, respectively. Their hair was cut from more than 1 cm from the scalp surface. Virgin black hair III was similarly obtained from a healthy 16-year-old girl of the Miao ethnic group living in the mountains of China's Yunnan province, and white hair IV from a 65-year-old Japanese male. All the hair fibers were straight, neither being stained with dyes nor subjected to any permanent setting processes. The hairs were successively washed with aqueous 1.5 wt.% aqueous sodium dodecyl sulfate (SDS), deionised water, and aqueous 70 vol.% ethanol; then the hairs were stored at 4°C in a sealed plastic container. All reagents, including 2-mercaptoethanol (ME), staining dyes, and papain, were commercially available.

PREPARATION OF THE SPECIMENS FOR OPTICAL MICROSCOPY[†]

The following four methods were adapted to prepare the specimens for microscopy. Methods 1–3 consisted of the chemical and enzymatic pretreatments of the hair fibers and the special cutting processes. Method 4 utilized a mini-vise to fracture the hair shaft.

Method 1: Simple swelling treatment of hair fibers by heating in the presence of urea and SDS (a general procedure). Hair (I–IV, 20 mg, about 1 cm length) was kept in the aqueous solution (5 ml) of 8 M urea and 4 wt.% SDS at 55°C for 5 h without stirring. Since the treatment solution did not contain ME, the hair shaft becomes soft without disturbance of the structure; see the swelling degree.[‡] The swollen shaft was washed briefly with pure water, dispersed in water (1 ml), and subjected to the cutting process I (vide infra). The resulting

[†]The benefits of staining of the dyes employed in the present study are as follows: CBB—The Mf of the Co and the Cu were nonspecifically stained blue. Congo red—In contrast with the Co, the Cu, particularly the blade-like shaped parts (CuB), were stained preferentially pale red. Gentian violet—This dye was milder than CBB, staining the cellular components in various depth of blue-violet. Giemsa—The Co, the handle-like shaped parts (CuH) of the Cu and the medulla's inner substance were more intensely stained than CuB and the medulla's wall. Silver—Almost of all proteinous substances were stained black. Silver staining was useful to recognize the presence of CuB that was hardly stainable with any of the organic dyes. SM—This dye has an Hg^{2+} ion in the molecule. Therefore, sulfur-rich cellular components or sites are preferentially stained in red (the color of the ligand) presumably due to a formation of a strong Hg–S linkage.

[‡]When hair (I–IV; about 20 mm length in plain water at 25°C) was heated in an aqueous mixture of 7 M urea, 3.5 wt.% SDS and 0 or 15 wt.% ME at 80°C for 20 min–1 h, the shafts were swollen in the following degree which was estimated using the equation, $100 \times (L_2 - L_1)/L_1$, where L_1 and L_2 are the breadth and length of the hair shaft before and after the treatment, respectively. On heating for 1 h in the absence of ME: (breadth) I 34 ± 6%, II 28 ± 4%, III 25 ± 7%, and IV 21 ± 8%; (length) I~IV about 0%. On heating for 20 min in the presence of ME: (breadth) I 143 ± 7%, II 150 ± 15%, III 150 ± 13%, and IV 200 ± 20%; (length) I 13 ± 4%, II 21 ± 2%, III 35 ± 2%, and IV 43 ± 3%. All the hair samples swelled maximally after heating for about 1 h: (breadth) I~IV 200 ± 15%; (length) I~II 40 ± 3%, and III~IV 50 ± 10%.

aqueous suspension of small hair fragments was mixed with a fresh 0.5 wt.% aqueous dye solution (2–3 drops) at ambient temperature for 0.5–5 h, where the dye used was Congo red, Coomassie brilliant blue G-250 (CBB), Gentian violet, 2',7'-dibromo-4'-hydroxymercurifluorescein sodium salt (SM), and a Giemsa's solution (Wako Pure Chem., Osaka, Japan) (16). Sometimes the hair fragments were silver stained by soaking in aqueous 0.2 wt.% silver nitrate, separating with a centrifuge, and exposing to bright sunlight for a while, followed by reduction with an aqueous 2 wt.% ascorbic acid at ambient temperature. After washing briefly with water, the stained sample was placed on a microscope glass slide using aqueous 50 wt.% glycerol as a mounting medium and overlaid with a cover glass (Matsunami Glass; No.1). A weight was placed on the cover glass (15–30 g/cm^2) while sealing the glass edges with Canada balsam.

Method 2: Treatment of hair fibers with papain (a general procedure). Hair (I–IV, 0.13 g, about 1 cm in length) was incubated with occasional shaking at 55°C for 2–6 h in the pH 7/0.07 M phosphate solution (10 ml) containing papain (crude powder type from *Carica papaya*, about 0.15 unit), L-cysteine (0.1 g, an activator), and SDS (0.23 g). The digested fibers were taken out with a spatula at 2-h intervals. After washing with water, the fibers were suspended in pure water (2.5 ml) and subjected to the cutting process I or III (vide infra). The resulting hair fragments were stained and mounted to a microscope glass slide in a manner similar to that mentioned above.

Method 3: Chemical treatment of hair fibers using ME (a general procedure). Hair (I–IV, 80 mg, about 2 cm in length) was put to an aqueous mixture (10 ml) of 6.4–8 M urea, 3–4 wt.% SDS and 1–20 wt.% ME in a screw-cap glass test tube. The tube was set in a horizontal position and warmed without shaking at 50°C–85°C for 20 min–12 h. After cooling to ambient temperature, the solid remainder of the hair shaft was washed briefly with aqueous 0.5 wt.% ME and subjected to the cutting process II or III (vide infra). The resulting suspension of hair fragments was centrifuged at 750 *g* to give the precipitate that was washed with aqueous 0.2 wt.% ME, stained, and mounted on the slide glass in a manner similar to that described above.

Method 4: Compression fracturing of the hair shaft. Hair (III, IV) was warmed in an aqueous solution of 7 M urea and 3.5 wt.% SDS at 55°C for 5 h. The resulting softened fiber was washed briefly with water, cut into about 10 mm in length, and 2 or 3 pieces were sandwiched between a glass slide (thickness, 1.3 mm) and a cover glass (20 × 20 mm with a thickness 0.72 mm) using Canada balsam as a medium. Subsequently, the glass plates were pressed by a mini-vise at the force which was slowly increased to about 0.5–1.0 kgf, taking about 15 min. The vise with the glass plates was then stored in a refrigerator (about 10°C) in order to harden the medium, followed by taking off the vise at ambient temperature to give the specimen for microscopic observation. By this compression method, the cuticular covering of the hair shaft was usually fractured in the same direction as the fiber's longitudinal axis.

MECHANICAL CUTTING PROCESSES (I, II, AND III)

The chemically and enzymatically pretreated hair fibers (obtained in the aforementioned methods 1–3) were randomly chopped using the following three kinds of cutters.

(i) A stainless steel gear (18 teeth, 8 mm diameter and 4 mm height) was placed in the aqueous suspension of the pretreated hair fibers at ambient temperature and rotated by a

small electric motor in the speed of 3500–4000 rpm for 3–10 s. This cutting method was found to effectively separate the blade-shaped parts (so-called "scale") from Cu.

(ii) A soft polyethylene gear having four teeth (each, 4 mm length × 2 mm width × 0.5 mm thickness) was rotated in an aqueous suspension of the pretreated hair fibers in water (3–5 fiber/ml) at the speed of about 500 rpm and ambient temperature for about 10 sec. This cutting process was used for the chemically or enzymatically pretreated hair fibers to coarsely chop the shaft.

(iii) A nose hair cutter (Hitachi, model BM-03, Tokyo, Japan) was used to obtain the very small hair fragments including the cells and cellular blocks. The outer steel blade with 9 slits (each slit: 0.8 mm width and 3.5 mm height) was designed to guide hair into a pair of inner blades (9 mm edge length, 2 mm depth, and 0.1 mm thickness). The pretreated hair fibers in distilled water (1.5–2.0 cm^3) were shortened with scissors to 1–5 mm length and put into the transparent plastic parabolic container (22 mm base diameter and 32 mm height), which was originally designed as the cap of the cutter head. The cutter body was

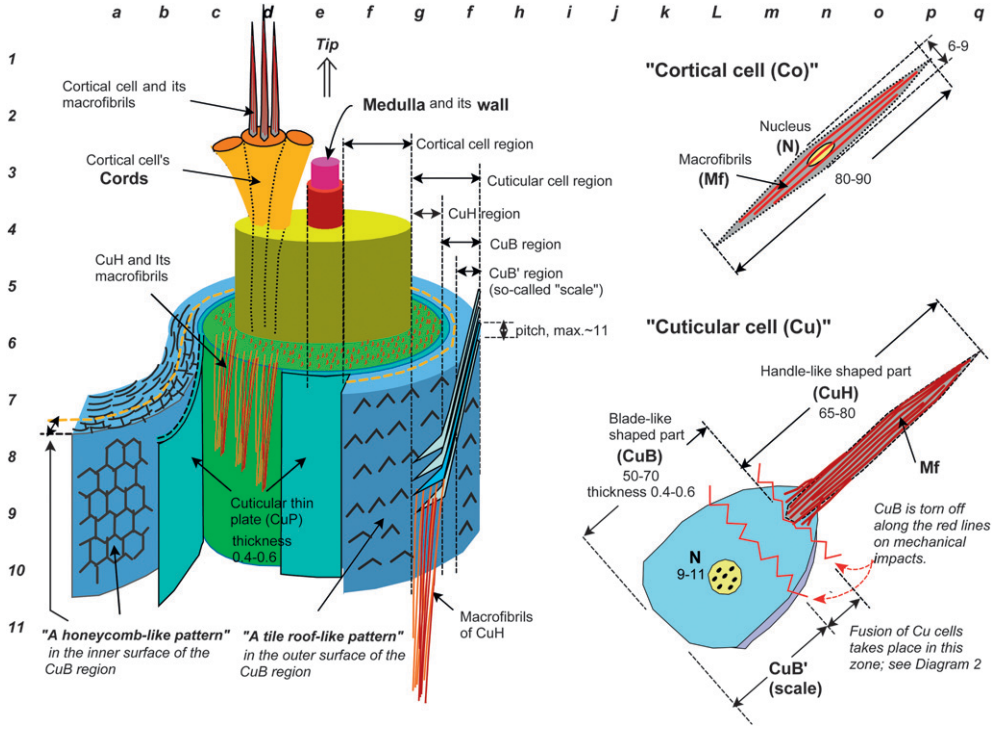

Diagram 1. Schematic representation of the structures of a human hair shaft, the cortical cell (Co) and the cuticular cell (Cu). Co takes a spindle-like shape. The cells gather together to form more than 20 thick cord-like shaped substances. Cu is a trowel-like shaped substance, consisting of a handle-like-shaped part (CuH) and a CuB. CuH is similar in dimensions to Co, and both are filled with plenty of macrofibrils (Mf). Cu overlap one another and fuse partially, displaying tile roof-like and honeycomb-like patterns in the outer and inner surfaces of the CuB region, respectively. CuB, in its basal area, merges completely with other units to produce the CuP, which encircles the inner cellular components. The nucleus (N) of Cu is in CuB. Medulla (M) is a tubular substance in the center of the hair fiber. The size (micrometer in unit) of the cells and the cellular components was measured by the use of an optical microscope and not corrected with the swelling degree of a hair shaft.[‡]

vertically clamped upside down, the container having the aqueous suspension of the fibers was set to the cutter head, and then the inner blade was rotated for 5–7 s.

MICROSCOPIC OBSERVATIONS

The biological microscopes, Olympus Photomax LB and BHS with proper attachments, were used for bright field, phase contrast, and polarized light observations. Objective lenses (Olympus) were as follows: (bright field) DPlanApo 10×, 20×, and 40×; CPL 20×/1.2; and LWDC Plan 40×/0.3–1.3. (Phase contrast) PL10×, PL20×, and PLL40×; (polarized) P20× and P40×; cf. the PlanApo lenses were usually used unless otherwise noted. Original photo tube accessories were modified to adapt the digital camera that was automatically controlled by a desktop computer to optimize for lighting, ISO levels (400-6400), and focusing measures. The images in JPEG and RAW formats were developed by means of Lightroom ver.3 and Photoshop Elements ver. 9 software (Adobe Systems Inc. San Jose, CA). Image enhancement included color level correction, noise reduction, and contrast and brightness adjustments; the purpose of the enhancement was to make the image appear nearly identical to that seen actually by the observer. In the case of thick specimens, the images taken at various depths of field were merged into a deep focus picture using the stacking software, CombineZM (A. Hadley, Sheffield, UK), (17).

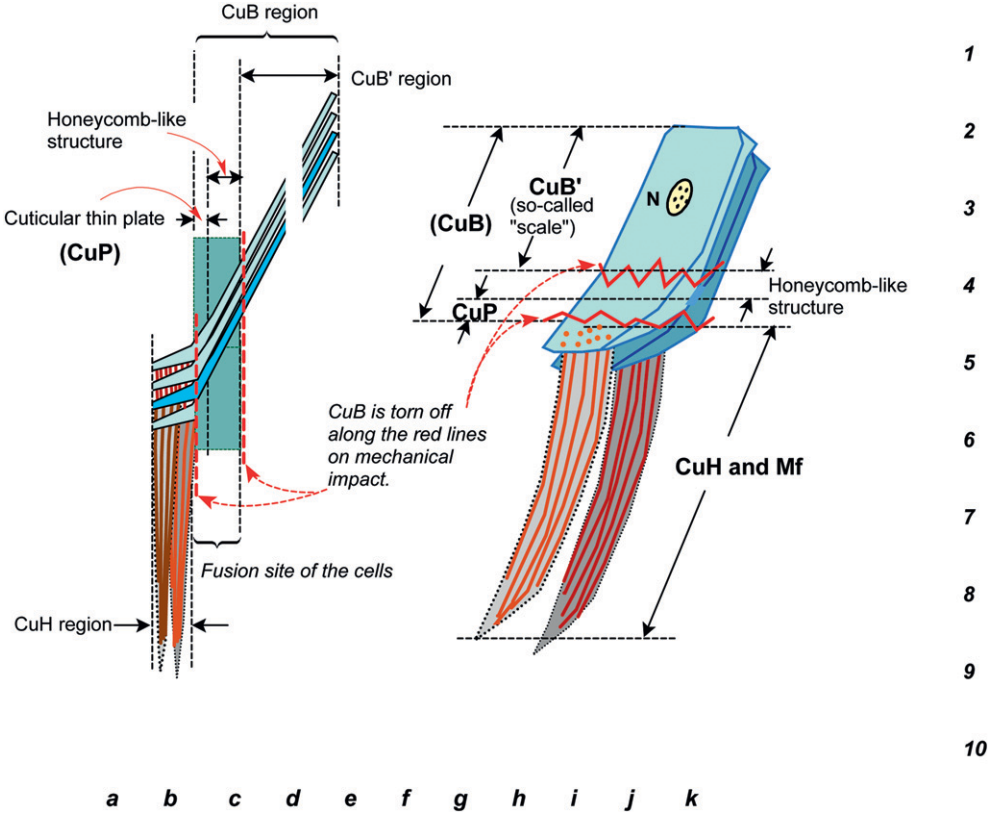

Diagram 2. Schematic representation of the side views of the Cu in a hair shaft. The cells overlap one another to form the honeycomb-like structure and the CuP. On the mechanical agitation, the cell is broken into the blade-like shaped part (CuB), the handle-like shaped part (CuH), and the fragmentary substance (CuB').

RESULTS AND DISCUSSION

In the present study, we examined the structure of the human scalp hair shaft, utilizing a methodology that was somewhat different from the previous investigations. The hair fibers were subjected to either the simple swelling treatment or the enzymatic proteolysis or the S–S bond cleavage reaction, followed by random scission using rapidly rotating cutters. The hair fibers were also physically fractured by strongly compressing the shaft with a vise. With these preparative methods, various kinds of specimens containing isolated

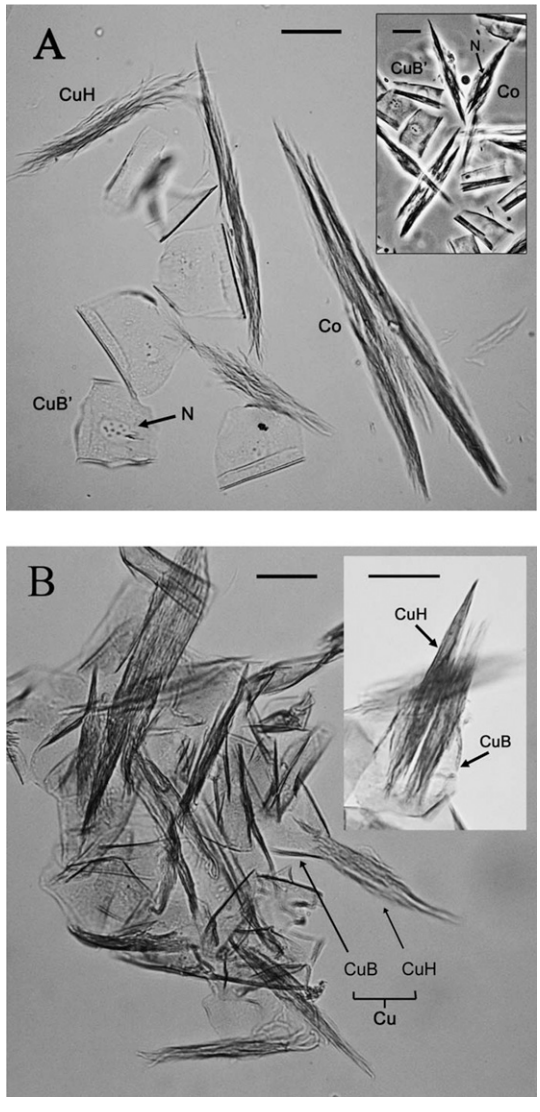

Figure 1. The photomicrographs of the Co and Cu including the cellular parts, PLL40× objective; bar 25 μm. Co, cortical cell; Cu, cuticular cell; CuB, the broad blade-like shaped part of Cu; CuB', the fragmentary substance derived from CuB; CuH, the handle-like shaped part of Cu; Mf, the macrofibrils of Co and Cu; and N, nucleus. Hair III was warmed in an aqueous solution of papain (0.15 unit), 1 wt.% L-cysteine and 2.3 wt.% SDS at 55°C for 3 h, then subjected to the cutting process II; Gentian violet staining.

cells, cells' blocks, fractured hair shaft, etc. were obtained. Like playing a jigsaw puzzle, the microphotographs of these disassembled hair parts were logically combined, resulting in the discovery of many unknown structures of the cellular components and the hair shaft itself. Some of them are schematically illustrated in Diagrams 1 and 2. The new structural features were common to all the hair samples (I–IV) examined.

CORTICAL CELL

The cell was easily recognized by a well-known spindle-like shape (a length of 80–90 μm and a width of 6–9 μm) as shown in Figure 1(A). The fibrous substances, which are most likely referred to as macrofibrils (Mf), were aligned in the same direction as the cell's longitudinal axis. Though the Cos, together with a medulla, have long been considered to occupy the inner domain of a hair shaft (1–3,12) and affect the fiber curvature (18–20), the cells were found to localize in the small space of the hair shaft, concentrically surrounding the medulla (M) (Figure 2). Furthermore, all the Cos appear to be grouped into more than 20 thick cord-like shaped substances [Figure 3(A)]. The substances twisted together as seen clearly in the specimens, which were prepared by physically fracturing the hair shaft [Figure 3(B) and 3(C)]. Formation of the cord-like shaped substances has not been explained well at present, but might be due to a special spatial arrangement of the Co; in other words, every Co was regularly shifted a little from neighbors along the long and short axes of the cell body (Figure 4). By the way, the Co was often more deeply stained with the basic dye, Gentian violet, than the Cu; the former cells were bluish purple while the latter, particularly the CuB part (vide infra), were gray or pale blue as shown in Figure 5. The facile stainability of the Co may be attributed to the high content of acidic amino acid residues of the keratin proteins involved (5,8,21).

CUTICULAR CELL

On chemical treatment with an aqueous mixture of urea, SDS, and ME, the hair shaft was transformed to a hollow fiber, gradually losing the inner substances [Figure 6(A) and

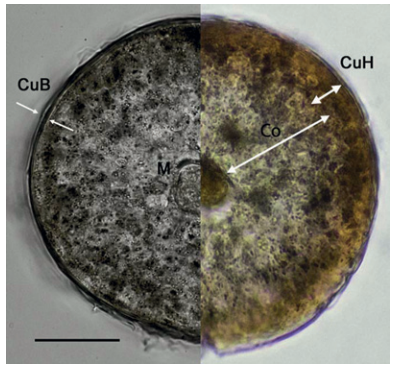

Figure 2. The cross section of a hair shaft (left: no staining; right: Congo red staining); bar 50 μm. The region of the handle-like shaped part (CuH) of the cuticular cells (Cu) was more deeply stained by the dye than the region of the cortical cells (Co). Hair II was heated in an aqueous solution of 7 M urea, 3.5 wt.% SDS and 15 wt.% ME at 55°C for 15–30 min, followed by slicing with the cutting process III. Thereafter, about half the amount of the resulting substances was subjected to Congo red staining.

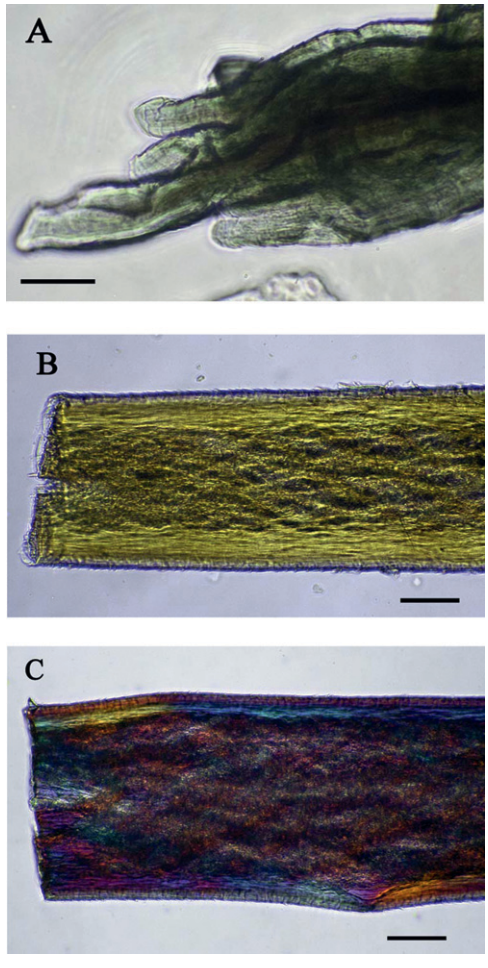

Figure 3. The cord-like shaped substances of cortical cells. (A) Hair III was treated in an aqueous solution of 8 M urea, 4 wt.% SDS and only 0.7 wt.% ME at 85°C for 2 h, then subjected to the cutting process II; CBB staining; PLL40× objective; bar 50 μm. (B and C) The polarized light microscopic pictures of the fractured shaft of a hair sample III, cf. the Method 4 in the experimental section; P40× objective; bar 50 μm. The hair fiber was optically anisotropic, especially in the direction of the longitudinal axis. Therefore, and the angle between the axis of the hair and the polarizer was varied properly while the angle between the polarizer and the analyzer was set to 45°; a sensitive tint plate was used. The chromatic distinction seen in the fractured hair may be chiefly caused by the difference in birefringence or the heterogeneity in the α-crystallites of the proteins involved.

6(B)]; cf. the footnote.[§] Amino acid analysis indicated that the hollow fiber was approximately comparable with the whole cuticles or so-called "cuticular scales" (Table I). The wall of the fiber chiefly comprised 7–12 overlapping scale-like substances [Figure 6(C)].

[§]The substance, which was squeezed out from hair shafts, was almost completely dissolved in the reaction medium, amounting to 45%–70% of the starting dry weight of hair shafts. On dialysis of the mother liquor, the keratinous proteins (MW 12–60 kD) were easily obtained as an aqueous solution (21,22). The amino acid analysis of the proteins was consistent with that of the Mf of the starting natural fibers. On oxidation treatment, the aqueous keratin solution was readily transformed to various water-insoluble biomaterials such as film (21,22,23), cultivation substrata (24), microcapsules (25), and sponge (26–28).

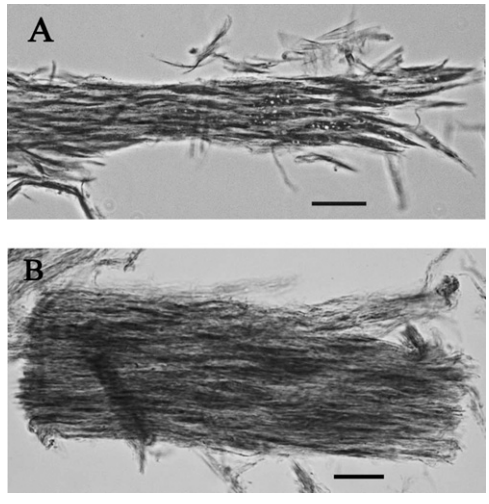

Figure 4. (A) A block of the cortical cells; PL20× objective; bars 25 μm. Hair I was heated in an aqueous mixture of 6.4 M urea, 3.2 wt.% SDS and 20 wt.% ME at 80°C for 7 h, followed by subjecting to the cutting process II; CBB-staining. (B) Plenty of the fibrous substance in the cortical cells' block; bar 50 μm. The specimen was prepared by treating hair III in a manner similar to that mentioned in panel A, except for Gentian violet staining.

The high tolerance of the wall to the severe chemical treatment may be attributed to the proteins that were heavily cross-linked with the isopeptide {ε-γ(glutamyl)lysine} and disulfide bonds (3–6 and 15–20 bonds per 1000 amino acid residues, respectively) (29, 30).

On the other hand, the Cu was found to take the trowel-like shape, consisting of a transparent CuB and a CuH; Figure 1(B) and the inset, cf. Diagram 1 (position: *10-m*). The spatial arrangement of CuB and CuH were judged in Figure 7, i.e., CuH was attached to the outer surface of CuB; cf. the schematic illustration in Diagram 2 (position: *6-b* and *5-i*). The cell itself, however, was not so stable on the mechanical impact given in the

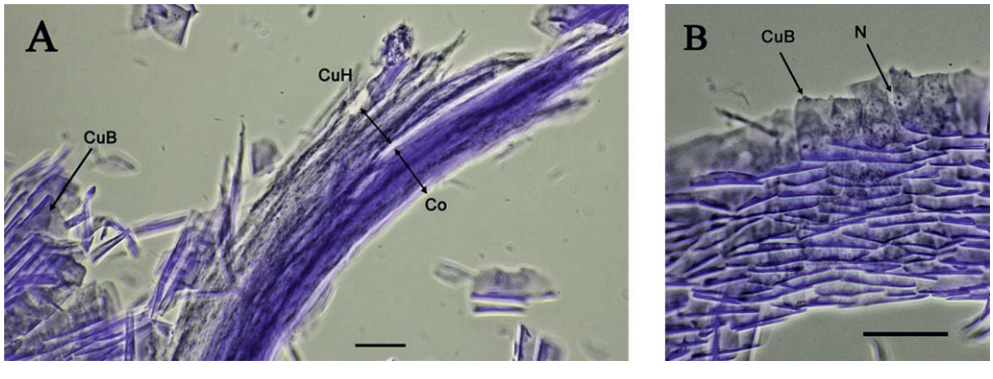

Figure 5. (A) Cortical cell (Co), the blade-like shaped (CuB), and handle-like shaped (CuH) parts of the cuticular cell; bar 50 μm; cf. Figure 1. (B) CuB overlapped the other to display a tile roof-like or so-called "scale" pattern; bar 50 μm. Every CuB possessed a nucleus (N) with 3–7 small black nucleoli. The specimens of panels A and B were obtained by heating hair (III) in a solution of 8 M urea and 4 wt.% SDS (without using ME) at 85°C for 15 h, followed by subjecting to the cutting process I; Gentian violet staining.

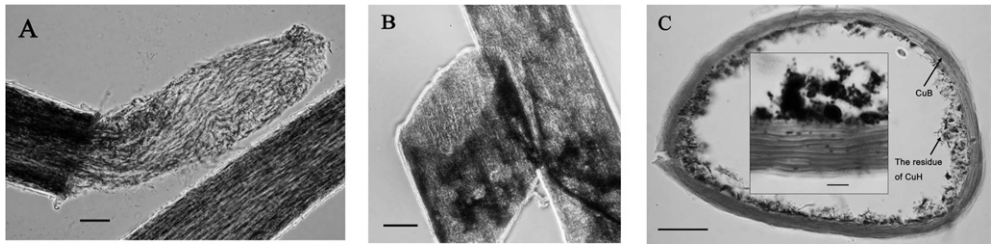

Figure 6. (A) The inner substances were squeezed out from hair (I) when the fiber was heated in an aqueous solution of 6.4 M urea, 3.2 wt.% SDS and 20 wt.% ME at 80°C for 2 h; staining with the Giemsa's solution; PL20× objective; bar 50 μm. (B) The hollow fiber; PL20× objective; bar 50 μm. Hair III was heated in 7 M urea, 3.5 wt.% SDS and 15 wt.% ME at 80°C for 7 h and subjected to the cutting process II; silver staining; (C) The cross section of the hollow fiber; bar 50 μm. Hair IV was heated in 7.6 M urea, 3.8 wt.% SDS and 5 wt.% ME at 80°C for 7 h, then subjected to the cutting process III; Gentian violet staining. The wall is mainly consisted of the blade-like shaped parts (CuB) of the cuticular cells. The black amorphous substances on the inner surface of the wall are most likely the remnant of the handle-like shaped part (CuH); cf. Figure 9(B). The inset is the enlarged view of a part of the wall; the small black spots may be melanin particles; bar 5 μm.

cutting process that it was split into CuB and CuH, and CuB was further broken into CuB'; Figure 1(A). CuB' was therefore recognizable as a small flat substance having at least one jagged edge, being very similar in shape to the traditional Cu (2,9); cf. Diagram 2 (position: *3-i*). On the other hand, CuH resembled the Co not only in shape but also in the richness of the fibrous substances (Mf) though the former part appeared to be more

Table I
Amino Acid Compositions of the Hollow Fibers and Whole Cuticle

	Residues per 1000 amino acid residues	
Amino acid	Hollow fiber from Hair III	Whole cuticle[a]
Cysteine + half Cysteine	155	180.8
Aspartic acid	54	32.4
Threonine	47	46.1
Serine	133	160.7
Glutamic acid	90	89.2
Proline	97	105.4
Glycine	93	88.4
Alanine	54	54.1
Valine	63	72.7
Methionine	1	4.6
Isoleucine	27	22.3
Leucine	52	44.6
Tyrosine	25	21.1
Phenylalanine	14	11.7
Lysine	39	34.4
Histidine	5	5.2
Arginine	36	26.4

[a]Cited from reference 7.

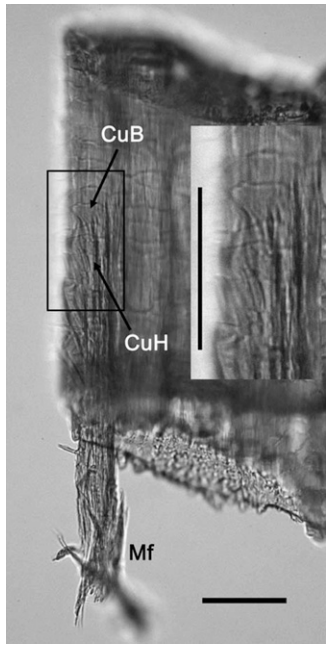

Figure 7. The hand-like-shaped part (CuH) of the Cu was attached on the outer surface of the blade-like-shaped part (CuB); bar 40 μm. The black line-framed area is enlarged to give the inset. The CuB in these pictures appears to be somewhat short in length, presumably because a tip portion of the complete form was shaved off in the cutting process. Hair IV was heated in 8 M urea and 4 wt.% SDS (without ME) at 60°C for 6 h, followed by the cutting process III; SM staining.

easily stained with Congo red than Co; Figures 1(A) and 2; cf. Diagram 1 (position: *2-m* vs. *8-m*). By the way, honeycomb-like and overlapping scale-like patterns were observed in the inner and outer surfaces of the CuB region, respectively; Figure 8; cf. Diagram 1 (position: *9-a* and *9-f*). A formation of these unique patterns is further discussed below.

CUTICULAR THIN PLATE

The present study indicated definitely the presence of a thin plate (CuP; 0.4–0.6 μm in thickness) in the Cu region; Figures 9(A), 9(B), and 10; cf. Diagram 1 (position: *9-e*) and Diagram 2 (*3-a* and *4-g*). CuP was a transparent material and its presence was revealed only by means of phase contrast microscopy; conversely, CuP was neither colored by polarized light microscopy nor stained with the dyes such as CBB and Gentian violet. When the infants' hair (I and II) were treated with the high concentrations of ME or for a long reaction time, CuP was isolated as a transparent film-like substance; Figure 9(C). Though the formation of CuP has not been explained well, it is conceivable that CuB fuse partially with the neighboring ones to build the honeycomb-like structure [Figure 8(A) and 8(B)], gradually merging into the single thin plate in the boundary zone between CuB and CuH. In fact, many merging sites were spotted in the inner surface of CuP; see, for example, the site marked by the gray circle of Figure 10(A). By the way, CuP was extremely tolerant to heating in the aqueous solution of 20 wt.% ME. The hollow fiber [Figure 6(B)] should not be stable without CuP; in other words, the hollow structure may be decomposed if the wall were merely constituted from the overlapping Cu. It would

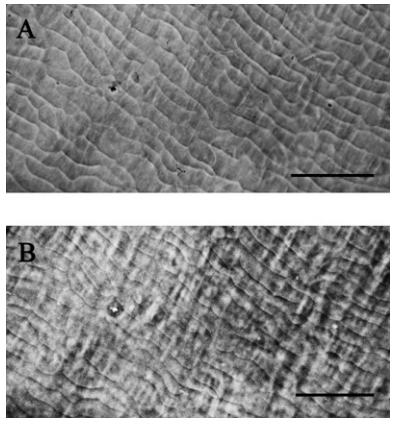

Figure 8. (A) A honeycomb-like pattern in the inner surface of the region of the blade-like shaped parts (CuB) of the Cu; PLL40× objective; bar 50 μm; cf. Diagram 1 (position: *9-a*). (B) A so-called "scale" pattern was seen when the reverse side of the CuB region (of the panel A) was focused on; note that the tiling direction was opposite between the panels A and B. The specimens of both panels were prepared by heating hair IV in an aqueous mixture of 7 M urea, 3.5 wt.% SDS and 15 wt.% ME at 80°C for 7 h, followed by the cutting process III; Gentian violet staining.

also be deduced that the inner components of hair including the Co and medulla are physicochemically protected, at least to some extent, from the outside circumstance by the presence of CuP.

MEDULLA

This component has been considered for a long time as a loosely packed assembly of the cellular disks connected in series (1,3,31). Nevertheless, it was suggested in the present

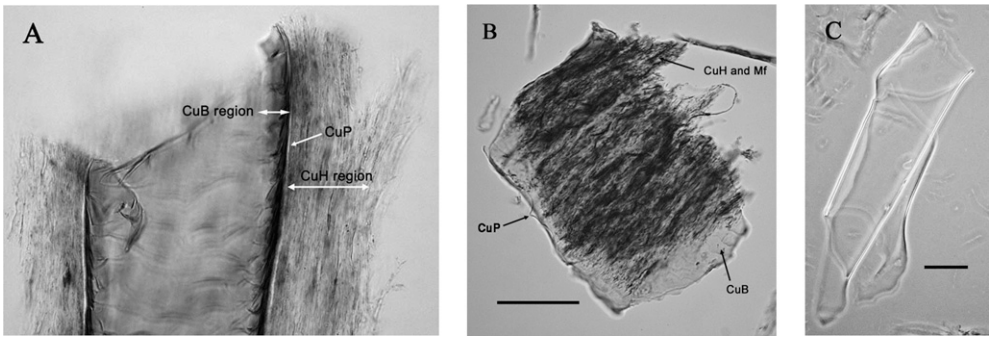

Figure 9. A cuticular thin plate (CuP) exists in between the regions of the blade-like shaped parts (CuB) and the handle-like shaped parts (CuH) of the Cu. (A) Hair III was heated in an aqueous solution of 7.4 M urea, 3.7 wt.% SDS and 8 wt.% ME at 50°C for 2 h and subjected to the cutting process II; CBB staining; PLL40× objective; bar 50 μm. As observed in this panel, the CuB region was usually paired with the CuH region presumably because both regions were linked to each other through CuP. (B) Part of the Cu region; PLL40× objective; bar 50 μm. CuP is seen as a transparent substance between the CuB and CuH regions in the phase contrast microscopy. The hair III was treated as mentioned in the panel A, except for the cutting process III and double staining with CBB and Gentian violet. (C) Part of an isolated CuP; PL20×; bar 50 μm. Hair II was heated in an aqueous mixture of 7 M urea, 3.5 wt.% SDS and 15 wt.% ME at 80°C for 3 h and subjected to the cutting process III; staining with Giemsa's solution.

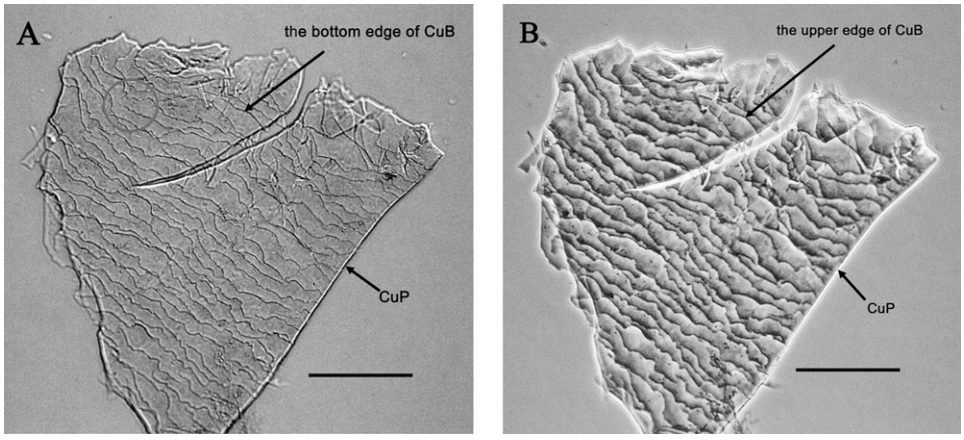

Figure 10. The honeycomb-like structure on the cuticular thin plate (CuP); PLL40× objective; bar 50 μm; cf. Figure 8. The pictures of the panels A and B were taken by focusing on the inner and outer surfaces of the plate, respectively. The gray circle in the panel A shows an example of the fusing sites of the blade-like shaped parts (CuB) of the Cu. The specimen was prepared by heating hair I in an aqueous mixture of 7 M urea, 3.5 wt.% SDS and 15 wt.% ME at 80°C for 2 h, then subjected to the cutting process III; staining with Giemsa's solution.

study that the medulla was a tubular substance with a wall-like coating; Figure 11; cf. Diagram 1 (position: *3-e*). It was also found that the medulla, like CuB, CuP, and hollow fiber, was stable to severe chemical treatments; for instance, heating in an aqueous solution of 20 wt.% ME at 50°C–80°C for 8–12 h. All of the hair samples (I–IV) showed that the medulla continuously streamed through in the center of the shaft. We have been investigating the physicochemical property of the medulla and will be reported later.

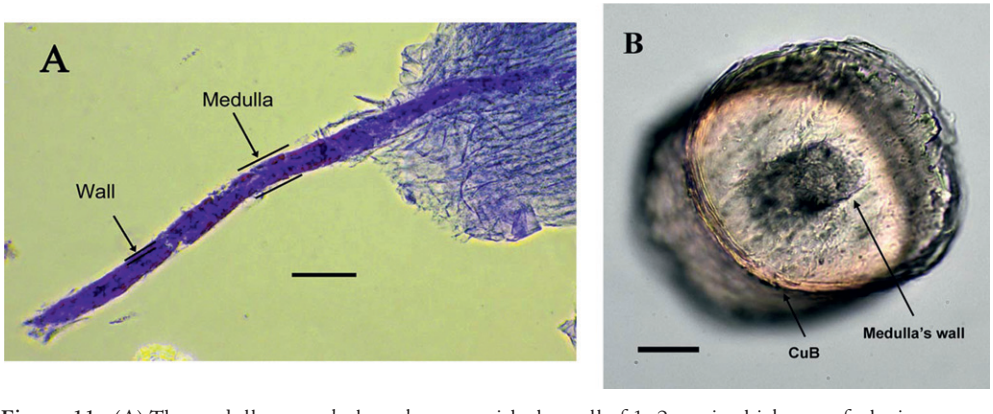

Figure 11. (A) The medulla as a tubular substance with the wall of 1–2 μm in thickness; cf. the image was enhanced using the color development software; PL20×; bar 30 μm. Hair III was heated in an aqueous mixture of 6.3 M urea, 3.4 wt.% SDS and 13 wt.% ME at 80°C for 6 h, then subjecting to the cutting process II; double staining with CBB and Giemsa's solution. (B) The medulla's wall and the blade-like shaped parts (CuB) of the Cu; bar 25 μm. It appears that the cortical cells and the handle-like shaped parts (CuH) were partially lost from the hair fiber during the preparation of the specimen. Hair IV was warmed in an aqueous mixture of 0.3 wt.% Congo red and 0.08 wt.% SDS (without ME) at pH 5 and 65°C for 30 min, washed with water, then subjected to the cutting process III.

CONCLUSIONS

The structure of the hair cells and the fiber shaft itself is substantially revised by optical microscopic observations of the hair components. Particularly, irrespective of the hair sources, that a mature Cu has a trowel-like shape with the following distinctive features: the part with handle-like shaped part (CuH) is full of Mf and usually colored by melanin granules, while the blade-like shaped parts fuses partially to build the honeycomb-like structure and a large cuticular thin plate. The medulla is tubular, streaming through all the hair shafts examined. The physicochemical stability of hair is mainly attributed to the presence of the honeycomb-like structure, the cuticular thin plate and the Mf of the Co and Cu. The present discoveries might be useful for people, especially chemists in the hair-related fields, to get new insights for their studies.

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