

Repairing Bleach-Damaged Hair by Treating With Polyphenol in the Presence of Cu (II) Ions

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Synopsis

This study first examined a quinone-crosslinking reaction by treating the model protein (lysozyme and water-soluble keratin) with various polyphenols (hydroquinone, catechol, chicoric acid, and others) in the presence of copper (II) chlorophyllin in an aqueous phase. The amino acid analysis, the SDS-PAGE, and the quantification of the SH group for the treated proteins suggested that the lysine, histidine, and cysteine residues of the proteins underwent cross-linking reaction with the polyphenols. Based on these findings, the bleached hair fibers were treated with the polyphenol in the presence of the copper salt (4 ppm). The resulting polyphenol-treated hair fibers were examined by means of optical microscopes (fluorescence and light-transmission modes) and an automatic tensile tester. It was found that the treatment of the bleached hair with chicoric acid in the presence of the copper salt was especially effective in repairing the hair damage, with respect to the cuticle scale-lifting, the swelling degree, and the mechanical properties (the breaking stress and initial elastic modulus). The present treatment was conducted under mild conditions and its potential application to hair-care technology for repairing bleached hair damage was briefly discussed.

INTRODUCTION

Many people today color their scalp hair using various dyes at home or in a beauty salon. The purpose of hair dyeing is mainly to modify the color of hair for one's liking or to make gray hair look inconspicuous.^{1,2} However, the aesthetic effects deteriorate with time, since hair grows from the root immediately after the treatment and the dyeing effect fades gradually due to hair washing. Because of this it is necessary to re-dye regularly. The repeated dyeing, as expected, gradually damages the physical properties of hair including tensile strength and texture.

Hair damage from dyeing and bleaching treatments is mainly caused by alkaline and oxidizing reagents, where the alkaline component assists the swelling and dyeing of the

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hair fibers and the oxidizing component mainly functions to decompose the melanin molecules—the coloring ingredient of natural hair. Through the treatments, a small portion of the keratin protein of the hair is changed to water-soluble products to escape from the fibers.³⁻⁵

Various repair methods have been proposed by cosmetic scientists to cope with these types of hair damage.⁶ Many of the methods aim to restore the physical properties of hair through by mending the damaged part of the hair using hair-care products.^{7,8} Some repair methods are similar to the processing technologies employed in the textile and food industries,⁹ such as a formation of complexes between proteins and phenols and tyrosine derivatives^{10,11} and a copper ion-catalyzed crosslinking reaction of gelatin with a quinone compound in an aqueous phase.¹² This study aims to explore a new hair treatment using a copper (II) ion-catalyzed cross-linking reaction with various polyphenols (Figure 1). The treated hair is evaluated mainly by the amino acid analysis, microscopic observations, and the mechanical characteristics.

EXPERIMENTAL

HAIR SAMPLES AND REAGENTS

Straight reddish black hairs (natural hair color) were kindly donated by Japanese women aged in their 20s and 30s. The hair fibers were neither bleached nor colored previously. The lysozyme from hen egg white (Sigma-Aldrich, Tokyo, Japan) and the water-soluble keratin (Keratec® IFP-HMW, Croda Japan KK, Shiga, Japan) were commercially available. Catechol, hydroquinone, and resorcinol were all reagent-grade (Sigma-Aldrich, Tokyo, Japan). Chicoric acid (Tokyo Kasei, Tokyo, Japan), copper (II) chlorophyllin (Cronon®

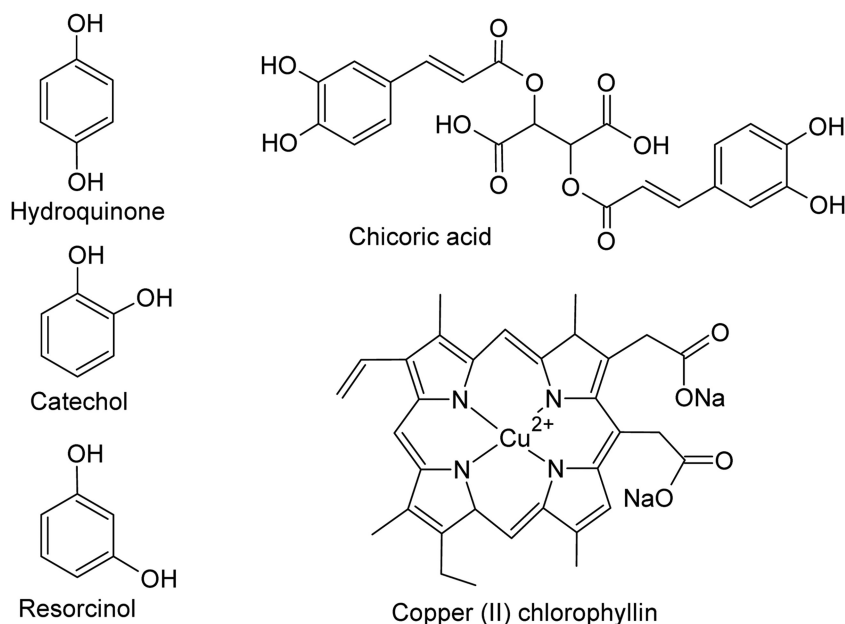


Figure 1. The polyphenols and the copper (II) chlorophyllin catalyst, which were used in this study.

G, Japan Chlorophyll, Saitama, Japan), Fluorescein-4-isothiocyanate (FITC-I) (Dojindo, Kumamoto, Japan), 2,2-Dithiobis(5-nitropyridine) (DTNP) (Sigma-Aldrich, Tokyo, Japan) and 10–20 wt% polyacrylamide gradient gel plates (Kishida Chemical, Osaka, Japan) were also commercially available. The bleaching was conducted using a mixture of Milbon Powder Bleach® (Milbon, Tokyo, Japan) and Ordeve Oxidane® 6.0 (Milbon, Tokyo, Japan); the former consisted of alkaline components and persulfate salt, and the latter contained 6 wt% hydrogen peroxide as an essential ingredient.

PREPARATION OF HAIR SAMPLES

Bleaching treatment. The previously mentioned bleaching and oxidizing agents were mixed in a ratio of 1:2 (w/w). After immersing the black hair in the aqueous mixture at a bath ratio of 1:50 (w/w) at 25°C for 30 minutes, the fibers were washed well with water and dried at 60°C with a hairdryer. These operations were repeated three times. The resulting hair will be from now on be referred to as bleached hair.

Cross-linking treatment of hair (a general procedure). The bleached hair fibers were immersed in pure water or a mixture of an aqueous solution of the polyphenol (8.4 mM) and copper (II) chlorophyllin (4 ppm) at a bath ratio of 1:50 (w/w) at 25°C for 12 hours. The resulting hair samples were rinsed with water and dried by using a hairdryer.

Cross-linking treatment of keratin proteins (a general procedure). The water-soluble keratin was diluted with the phosphate buffer (PBS) (0.1 M, pH 7.4) to 5 wt%. Similarly, the PBS solution of polyphenol (hydroquinone, catechol, resorcinol and chicoric acid) was prepared at the appropriate concentration. The reaction was started by adding an aqueous solution of copper (II) chlorophyllin (0.060mL) and the polyphenol solution (0.24mL) to the protein solution (2.7mL). After standing still at 40°C for 12 hours, the reaction mixture was obtained. In control experiments of the reaction, the keratin was replaced with an equal volume of lysozyme (0.8 wt%).

SODIUM DODECYL SULFATE POLY(ACRYLAMIDE) GEL ELECTROPHORESIS (SDS-PAGE)

The SDS-PAGE was carried out in a manner similar to a previous method.¹³ The polyphenol treated keratin solution (10 µL) was diluted with the X2 Laemmli buffer (Bio-Rad Laboratories, Hercules, California, USA) (10 µL). Each of the mixtures was heated at 100°C for 5 minutes, cooled to ambient temperature and applied to the polyacrylamide gradient gel plate (10–20 wt%). The resulting gel plate was subjected to electrophoresis at 20 mA for about 90 minutes. The protein molecular weight maker II (TEFCO, Tokyo, Japan) was also electrophoresed at the same time. The protein bands were visualized by staining with Coomassie brilliant blue (CBB).

AMINO ACID ANALYSIS

The amino acid analyses of the polyphenol treated keratin and hair were performed according to a high-speed RP-HPLC/FL method.^{14,15} Polyphenol treated product (1mg) was hydrolyzed in the vapor phase with 6 M HCl at 110°C for 24 hours according to the PicoTag protocol (Waters Corporation, Milford, Massachusetts, USA). The resulting hydrolysate

was assayed for an amino acid distribution by means of a fully automatic NEXERA XR system amino analyzer (Shimadzu, Kyoto, Japan) equipped with the separation column (150mm x 3.0mm) packed with 3.0 μ m diameter YMC-Triart C18 beads (YMC Co., LTD, Kyoto, Japan). Solution A (10 mM Na_2HPO_4 , 10 mM $\text{Na}_2\text{B}_4\text{O}_7$, pH 8.2) and B (acetonitrile : MeOH : H_2O = 45 : 45 : 10 v/v) were used as a mobile phase.

QUANTIFICATION OF THIOL RESIDUE OF KERATIN

The thiol residue was quantified according to the method adapted by Obata et al.¹⁶ A mixture of the keratin (1 wt%), chicoric acid (4.0 mM) and copper (II) chlorophyllin (4 ppm) was dissolved in phosphate buffer (0.1 M, pH 7.4) and warmed at 40°C for 12 hours. The resulting reaction mixture was diluted by a factor of 10 $\times$ with PBS and dialyzed against the same buffer at 4°C for 12 hours using a membrane tubing (molecular weight cut-off of 14.0 kDa). Subsequently, the dialyzed solution (0.4mL) was mixed with an ethanol solution of DTNP (0.5 mM, 0.1mL) and warmed at 37°C for 30 minutes, then was subjected to the absorption measurement at 386 nm using a U-3310 UV-spectrometer (Hitachi, Ibaraki, Japan). The number of thiol residue in keratin protein was calculated with the calibration curve, which was obtained using L-cysteine as a reference compound; viz. the cysteine concentration was well correlated to the absorbance at 386 nm by equation; (absorbance at 386 nm) = cysteine concentration (μM) $\times$ 0.0092 + 0.3144.

FLUORESCENCE MICROSCOPIC OBSERVATIONS OF FITC-I DYEING HAIR SAMPLE

The fluorescence measurement was carried out according to the procedure reported by Nakamura et al.¹⁷ Several strands of hairs were added into carbonate buffer (0.1 M, pH 5.0) containing FITC-I (180 ppm) and left for 12 hours. The resulting hair fibers were washed three times with 0.5mL of carbonate buffer (0.1 M, pH 9.5) and sliced cross-sectionally by means of a microtome to obtain the specimens (about 20 μ m in thickness), then subjected to the fluorescent measurement at excitation wavelength of 470–495 nm and fluorescence wavelength of 510–550 nm using a Olympus BX51 fluorescence microscope (Olympus Corporation, Tokyo, Japan) equipped with a U-MNIBA3 mirror unit (Olympus Corporation, Tokyo, Japan). The shutter speed was 2.5 seconds and the ISO sensitivity was 800.

OPTICAL MICROSCOPIC OBSERVATIONS FOR EXAMINATION OF POLYPHENOL-TREATMENT OF BLEACHED HAIR

Biological microscopes (Olympus Photomax with the proper attachments) were used for bright field observations (Olympus Corporation, Tokyo, Japan). The Olympus objective lenses were: (bright field) Plan $\times$ 10, PlanApo $\times$ 20 and PlanApo $\times$ 40 (Olympus Corporation, Tokyo, Japan). 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 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 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.

The specimens for the optical microscope were prepared in the following manner: the natural hair, the bleached hair and chicoric acid, and the copper (II) chlorophyllin treated bleached hairs, were cut to about 10mm in length. About 30 strands of the shortened sample were then immersed in aqueous 5 M urea and warmed at 60°C for 1 hour. After cooling to ambient temperature, 10 strands of the swollen sample were mounted parallel to a microscope slide glass, where the medium was aqueous 5 M urea. No weight was placed on the micro cover glass (22 mm × 22 mm; thickness, 0.13–0.17 mm) (Matsunami Glass, Osaka, Japan) while sealing the edges with UV Curable Urethane Acrylate Oligomer (Mitsubishi Chemical, Tokyo, Japan) and irradiating with a 365 nm LED-light for 3 minutes at ambient temperature.

MEASUREMENT OF SWELLING DEGREE OF HAIR FIBER

Various hair fibers (about 15mm in length) were warmed in aqueous 5 M urea solution at 60°C for 1 hour. The swollen fiber in the solution was kept at ambient temperature for 1 day, then gently placed on the slide glass with a cover glass using 5 M urea solution. The cross-sectional length (L_s) was measured by means of the optical microscope using the Plan ×20 objective lenses. The swelling degree (length-%) was calculated by $100 \times (L_s - L_d) \div L_d$, where L_d is the cross-sectional length of the fiber before swelling. 7 strands of the same hair sample were tested to average the swelling degree.

TENSILE TESTING OF HAIR SAMPLES

The polyphenol treated fibers (20–100 strands) were immersed in purified water for 12 hours, and then set in an ALS1500 + MTT690 automatic tensile tester (Dia-Stron, Hampshire, UK). Tensile measurement was performed at pulling speed of 3mm/min while immersing the hair sample (30mm in length) in water. Under the testing condition, which was similar to ones used previously,^{18,19} we experienced that the tensile data was obtainable with small dispersion. 60 fibers per test cell ($N = 60$, 3 persons, 20 fiber samples each) were individually applied to the tests for estimating the mechanical properties (the breaking stress, the breaking extension, and the elastic modulus in the initial pilling stage). The breaking stress (J/m^3) applied to breaking total work (J) per the volume of sample hair ($(d \div 2)^2 \times \pi \times L_0$). In the denominator, d is an average value of a hair diameter at 5 points as measured with a laser contour measuring device (FDAS770, Dia-Stron; Hampshire, UK). L_0 is the initial hair length. The breaking extension (%) is given by $100 \times (L - L_0) \div L_0$, where L is the breaking length of hair specimen. The initial elastic modulus (J/m^3) was the Hookean slope of at the 3% extension.

RESULTS AND DISCUSSION

POLYMERIZATION OF THE POLYPHENOL TREATED PROTEINS

This study was first conducted to examine an action of various kinds of polyphenols (0.9 mM) on lysozyme (0.28 mM, MW: 14,300), a model protein, in the presence of a catalytic concentration (4 ppm) of copper (II) chlorophyllin (Figure 1). Figure 2 shows the SDS-PAGE pattern of the reaction mixtures. When the model protein was treated

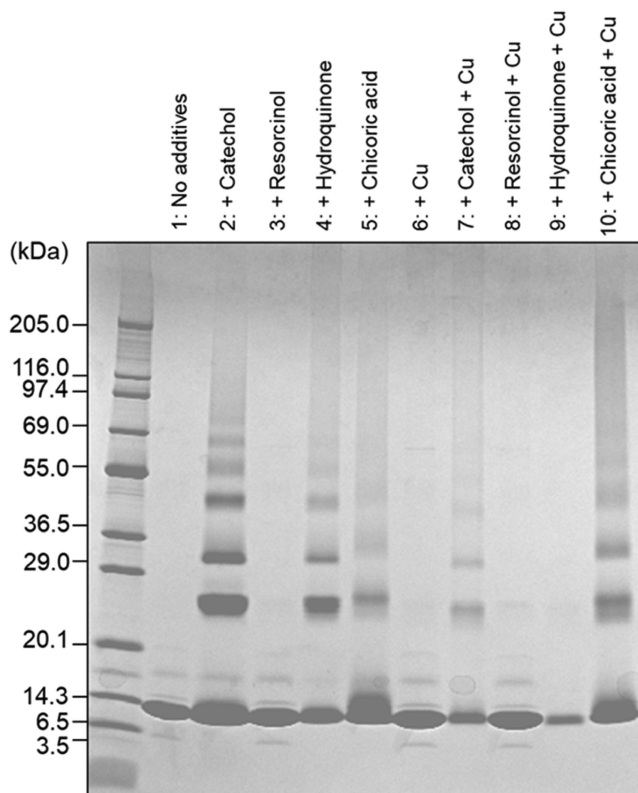


Figure 2. SDS-PAGE patterns of the lysozyme (0.4 wt.-%) which was treated with various polyphenols (0.9 mM) in the presence or absence of copper (II) chlorophyllin (Cu, 4 ppm) at 40°C for 24h. CBB was used as a staining agent. It is considered that the very large proteins products, if any, were retained in the starting wells without running through the gel.

with catechol, hydroquinone, chicoric acid, and resorcinol, the former three polyphenols showed ladder-like lanes which ranged from about 25–70 kDa as seen in lanes 2, 4, and 5, respectively. By contrast, resorcinol was not active for increasing the molecular weight distribution of the protein; see lane 3.

It was also found that a combination of the polyphenols with the copper (II) chlorophyllin (often referred to hereafter as copper salt) was highly effective in increasing the molecular weight-range of the lysozyme; see Figure 2 and lanes 7, 9, and 10. In other words, the molecular weight-range of the protein was significantly increased when the protein was treated with catechol, hydroquinone, and chicoric acid. Although the reaction using hydroquinone and the copper salt just showed only faintly stained medium molecular weight substances (25–120 kDa) (lanes 7 and 9), the product was considered as the highly polymerized proteinous substances separated from the buffer medium did not run through the gel disk. A similar electrophoresis phenomenon was observed in other systems using gelatin proteins.¹² Although the detailed analysis of the catalytic role of the copper salt is not described here, the optimal concentration of the copper salt was around about 4 ppm. The salt concentration was therefore employed throughout this study.

Figure 3 is the SDS-PAGE band pattern of the reaction products which were obtained by treating lysozyme (0.4 wt.-%) with various concentrations of the polyphenols (hydroquinone and chicoric acid) in the presence of the copper salt (4 ppm). At the low hydroquinone concentration (0.9 mM), a well-defined ladder-like lane was clearly observed. In the higher concentration (4.5–36 mM), however, not only lysozyme (ca. 14 kDa) but also the protein ladder (25–210 kDa) disappeared. Like the previous results, it is likely that high molecular weight and insoluble products were not run through the SDS-PAGE gel. However, chicoric acid was a relatively milder reagent to provide the polymerized protein bands in a wide concentration range (0.3–12 mM), showing well-defined ladder-like patterns in the medium molecular weight-range.

Figure 4 shows the SDS-PAGE pattern observed by the keratin (5 wt.-%) with the polyphenol (hydroquinone and chicoric acid) in the presence of the copper salt (4 ppm) at 40°C for 24 h. With hydroquinone, the broad band (about 6–120 kDa) of the starting keratin was disappeared while the new band (about 80–350 kDa) appeared, particularly in the low concentration (9.0–0.9 mM). It seemed, similar to the lysozyme, that very large molecular weight-substances were insoluble and retained in the electrophoresis-wells without flowing through the gel. In the case of chicoric acid, the product bands were clearly observed and steadily increased with the increasing concentration of the polyphenol. It appears that the activity of chicoric acid at 6.0–12 mM concentration was comparable to that of 0.9–4.5 mM hydroquinone. The previously mentioned electrophoresis data suggested that the polyphenols act on the proteins to polymerize to the higher molecular weight substances and that the hydroquinone and chicoric acid were especially effective in promoting the polymerization reaction.

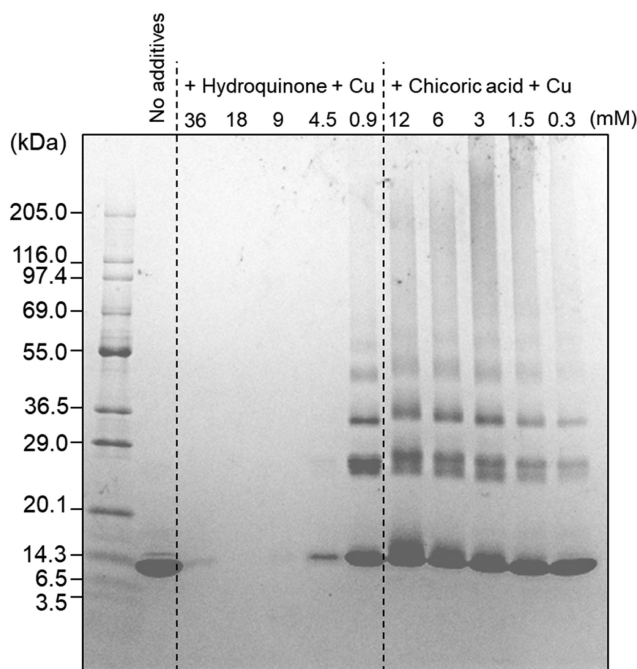


Figure 3. SDS-PAGE patterns of the lysozyme (0.4 wt.-%) which was treated with various concentrations (mM) of hydroquinone or chicoric acid in the presence of copper (II) chlorophyllin (4 ppm). CBB was used as a staining agent.

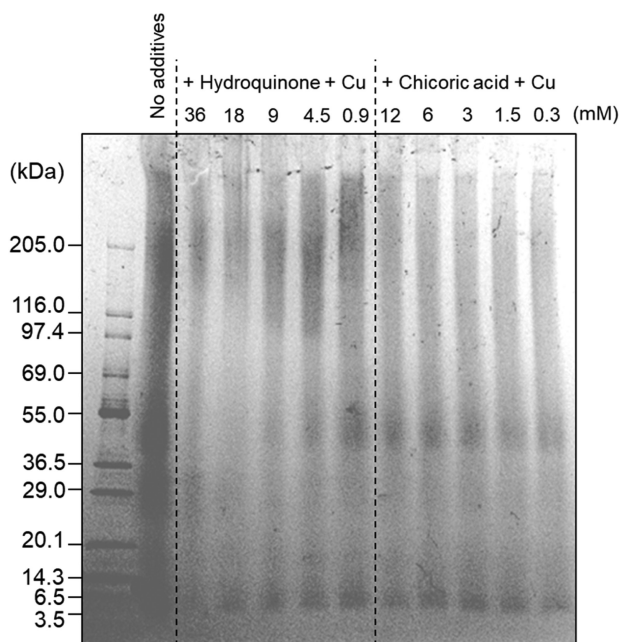


Figure 4. SDS-PAGE patterns of the keratin protein (IFP-HMW) which was treated with the polyphenol (hydroquinone and chicoric acid) in the presence of copper (II) chlorophyllin (4 ppm). The concentration (mM) of the polyphenols is indicated on the upper side of the lanes. CBB was used as a staining agent.

CHEMICAL CHARACTERIZATION OF THE POLYPHENOL TREATED HAIR FIBERS

We next assayed the amino acid residues of the water-soluble keratin and the bleached hair which were treated with chicoric acid in the presence of the copper salt (4 ppm). Table I lists the amino acid compositions before and after the treatment. The lysine, histidine and cysteine residues were substantially decreased in both the keratin protein and the bleached hair after the treatment; see the three residues from the bottom of Table I. Also, the UV-spectrometric quantification clearly indicated that the cysteine content of the protein was decreased to about one-fifth level treatment with the polyphenol and the copper salt, as shown in Figure 5. These results of the SDS-PAGE and the amino acid analysis suggest that the lysine, histidine, and cysteine residues were chiefly modified with the polyphenols in the presence of copper (II) ions to polymerize the keratin proteins. Similarly, Yamauchi and coworkers studied an artificial sclerotization of gelatin to conclude that these amino acid residues reacted with hydroquinone, catechol, and others to rapidly harden the structural protein in the presence of a catalytic concentration by copper (II) ions.¹²

On the other hand, Figure 6 shows cross-sectional photographs of untreated hair, the bleached hair, and the chicoric acid treated bleached-hair, each of which was stained with the fluorescent dye (FITC-I) in the same conditions. Note that the dye generally reacts with the lysine, histidine, and cysteine residues of a protein.²² The untreated hair gave the weak fluorescence only on the thin surface of the fiber as seen in Figure 6A. The bleached hair was stained more intensity with FITC-I, especially in the cuticle region and the medulla regions,²³ which were abundant with lysine and cysteine residues (Figure 6B).²⁴

Table I

Effects of the Treatment with Chicoric Acid and Copper (II) Chlorophyllin on the Amino Acid Compositions of the Keratin Protein and the Bleached Hair^a

	Keratin Protein	Chicoric acid treated keratin protein (The rate of change by the treatment)		Bleached hair	Chicoric acid treated bleached hair (The rate of change by the treatment)	
Asp(Asn)	63.1	63.1	(1.0)	56.4	56.4	(1.0)
Glu(Gln)	113.6	113.9	(1.0)	106.2	111.1	(1.0)
Ser	83.8	82.0	(1.0)	91.9	94.5	(1.0)
Gly	62.8	58.6	(0.9)	50.1	49.5	(1.0)
Thr	53.7	54.2	(1.0)	62.0	66.1	(1.1)
Arg	64.2	62.1	(1.0)	57.7	58.6	(1.0)
Ala	48.6	48.3	(1.0)	38.6	40.3	(1.0)
Tyr	20.0	18.2	(0.9)	17.7	18.0	(1.0)
Val	43.4	46.1	(1.1)	39.6	40.6	(1.0)
Met	3.3	3.0	(0.9)	4.3	4.3	(1.0)
Phe	22.7	22.8	(1.0)	15.4	14.0	(0.9)
Ile	29.6	30.4	(1.0)	21.8	22.9	(1.1)
Leu	73.1	73.7	(1.0)	49.0	46.8	(1.0)
Pro	68.7	62.7	(0.9)	69.3	61.2	(0.9)
His	4.0	2.0	(0.5)	7.8	5.1	(0.7)
Lys	20.6	11.9	(0.6)	23.3	13.7	(0.6)
Cys ^b	224.6	152.2	(0.7)	288.9	180.7	(0.6)
Total	1000	1000		1000	1000	

- (a) The keratin protein was treated with chicoric acid (3.6 mM) in the presence of the copper salt (4 ppm). The bleached hair fibers were treated with chicoric acid (8.4 mM) in the presence of the copper salt (4 ppm). Total amino acid content was assumed to 1000 residues. Tryptophan was not observed.
- (b) A combined total of the cysteine and half-cysteine contents. The sum was not significantly altered between different runs of the treatment. By contrast, the individual contents for the amino acid residues were substantially varied, presumably owing to a complex redox action of the polyphenol-quinone system in the present treatment.^{20,21}

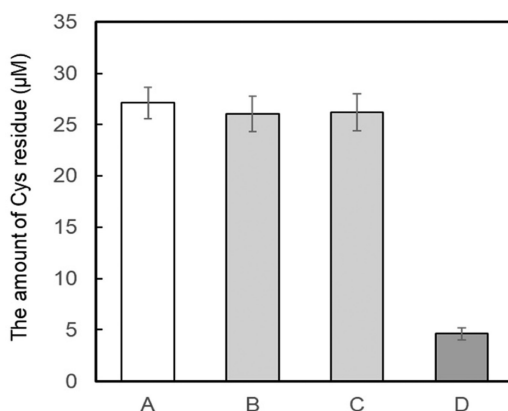


Figure 5. The UV-spectroscopic quantitative assay of the cysteine residue of the keratin protein which treated under various conditions. (A) No treatment; (B) only chicoric acid; (C) only copper (II) chlorophyllin; (D) chicoric acid and the copper salt. See the experimental section for the spectroscopic quantification of the thiol group.

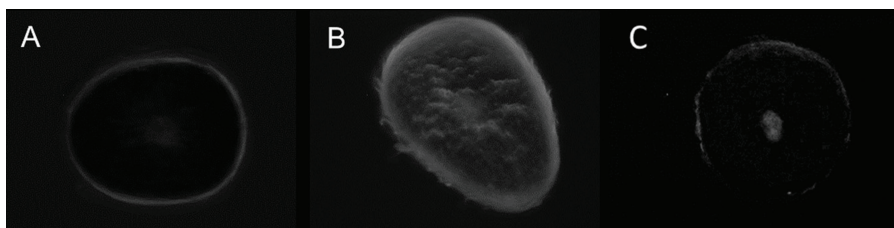


Figure 6. Fluorescence micrographs of the cross-section of various hair fibers which were stained with FITC-I. (A) Untreated natural hair; (B) bleached hair; (C) bleached hair treated with chicoric acid (12 mM) in the presence of copper (II) chlorophyllin (4 ppm). See the experimental section for further details.

By contrast, chicoric acid, and the copper salt treated bleached-hair became much less fluorescent as displayed in Figure 6C. The weakened fluorescence by treatment with chicoric acid may be attributed to the decreased binding sites in the hair toward FITC-I, or the aforementioned three kinds of amino acid residues were decreased in content upon binding with chicoric acid.

Based on the previously mentioned results, it is suggested that the lysine, histidine and cysteine residues in the model proteins and the bleached hair underwent a cross-linking reaction by the action of the polyphenols in the presence of the catalytic concentration of the copper salt. The reaction may be similar to that of the metal ion-catalyzed hardening of gelatin with polyphenols.¹² The tentative reaction pathways are displayed schematically in Figure 7A.

MICROSCOPIC OBSERVATIONS OF THE POLYPHENOL TREATED BLEACHED HAIRS

We also examined the swelling degree and the scale lifting of various hair fibers using the optical microscope. The specimens were: the natural hair (I), the bleached hair (II), the bleached hair treated with the copper salt (III), the bleached hair treated with chicoric acid (IV), and the bleached hair fibers treated with chicoric acid in the presence of the copper salt (V). The swelling degree of specimens I–V was measured upon warming in aqueous 5 M urea at 60°C for 1 hour to give the following results: I: $31 \pm 5\%$; II: $45 \pm 6\%$; III: $61 \pm 10\%$; IV: $44 \pm 3\%$; V: $26 \pm 3\%$. It is worth noting that specimen V, which was treated with chicoric acid and the copper salt, was not swollen as much as I (natural hair), and its swelling degree was the lowest of that of the other specimens.

It was also observed that specimens II–IV underwent substantial scale lifting upon warming in 5 M urea. See the representative photomicrograph of Figure 8A versus 8B, 8C and 8D; note the native hair (I) resisted even a medium lifting of the scales. The treatments of the swollen bleached hair with the copper salt alone (III) and with the polyphenol alone (IV) were not appreciably affect the scale lifting. By contrast, the scale lifting of the bleached hair was markedly subdued by treating with the polyphenols in the presence of the copper salt; see Panel E. The swelling degree and the scale lifting were reproducibly observed by employing 7 strands and 50 strands of each specimen, respectively. Therefore, the bleached hair was considerably repaired with respect to the swelling degree and the scale lifting when treating with a system of chicoric acid and the copper salt.

Specimen (V), which was obtained using chicoric acid and the copper salt, was visually characterized with light brown color. The other specimens (II–IV) were all colored in tint

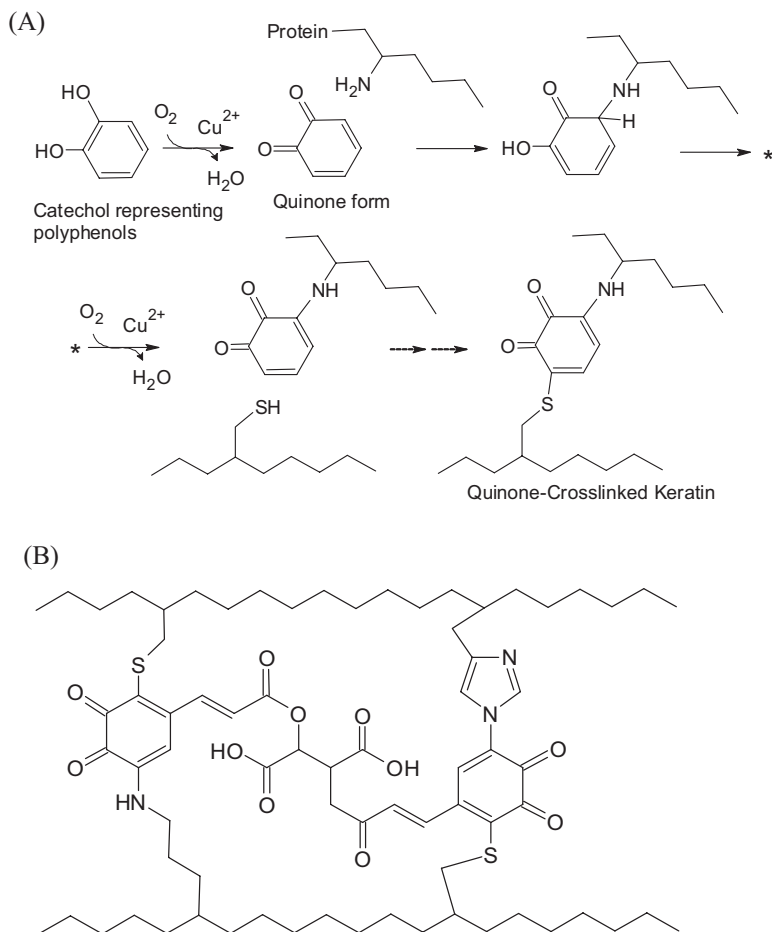


Figure 7. (A) Tentative pathways for the copper (II) ion-catalyzed cross-linking reaction of proteins. A formation of the quinone-crosslink between lysine and cysteine residues is shown as an example. The polyphenol is oxidized by oxygen to the quinone form. The copper (II) works as a catalysis in the oxidized reaction. The carbonyl group of the quinone is then attacked by amines or thiol groups of the protein in a nucleophilic fashion to bind to the protein; see the text for further details.¹² (B) An example of the crosslinked proteinous substance derived using chicoric acid.

yellow. A coloring phenomenon was also observed in the artificial sclerotization of gelatin with various polyphenols in the presence of copper (II) ion catalysis.¹² We believe that a small portion of the polyphenol adsorbed on the hair underwent oxidative polymerization to form complex polyphenolic byproducts to stain the fiber shafts.

Hair damage and scale lifting with chemical oxidize bleaching and under alkaline condition were discussed.¹ We have currently speculated that the decreased swelling degree and the diminished extent of the scale lifting, especially in the hair specimen (V), was due to the aforementioned copper (II) ion-catalyzed polyphenol-crosslinking reaction in the keratinous proteins of the cuticular cell regions,²³ although the exact reaction sites have not been specified yet.

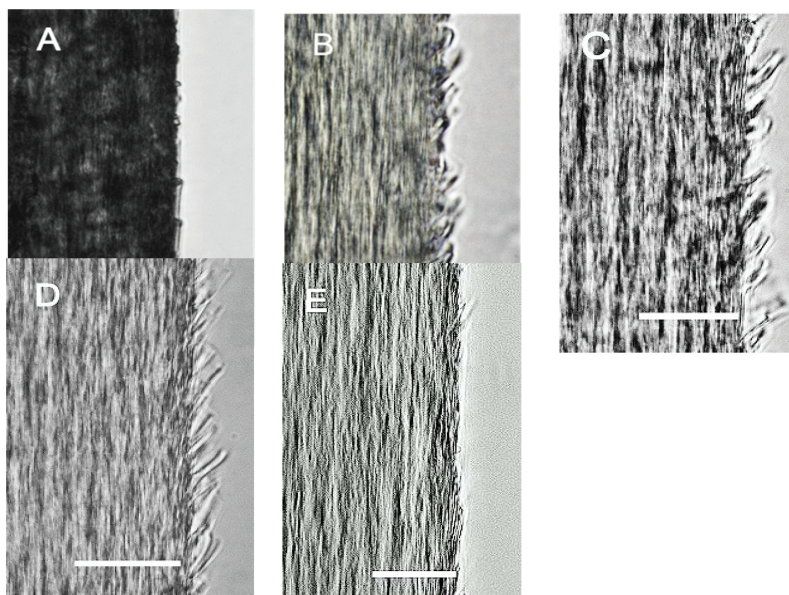


Figure 8. Optical microscopic observations under a transmission illumination mode on the various hair samples which were warmed in aqueous 5 M urea solution at 60°C for 1h. The scale bar is 30 μm . (A) Natural hair (specimen I); (B) bleached hair (specimen II); (C) the copper salt treated bleached hair (specimen III); (D) chicoric acid treated bleached hair (specimen IV); (E) chicoric acid and the copper salt-treated bleached hair (specimen V).

TENSILE PROPERTIES OF THE POLYPHENOL-TREATED HAIR FIBERS

We next investigated the tensile properties of various kinds of the hair specimens in water. Figure 9 displays the tensile properties of the natural hair and the bleached hair, which were treated with various polyphenols in the presence of copper (II) chlorophyllin. As anticipated, the bleaching treatment weakened the fiber, resulting in decreasing both the initial elastic modulus and the breaking stress. The decreased mechanical properties may be attributed to the decreased number of disulfide bonds in the intermediate filaments,²⁵ presumably by an oxidative action of hydrogen peroxide.²⁶⁻²⁹

On the other hand, the combination of polyphenols and the copper salt was very active in improving the tensile properties of the bleached fiber, except resorcinol, which did not function as a crosslinking agent (Figure 2, lane 8). This means that catechol, hydroquinone, and chicoric acid were comparable in increasing the breaking strength and the elastic modulus (low initial stiffness); see Figure 9A and 9B. The breaking extension of Figure 9C, in contrast, showed no significant differences among hair samples (the natural, untreated bleached hair and treated bleached hair fibers). It is remarkable that chicoric acid was especially effective for repairing the mechanical strength of the bleached hair into a comparable level of the natural hair. Although copper (II) ion itself has also been reported to form crosslinks at cysteine residues,³⁰ the concentration of the metal ion (4 ppm) used in the present study was very low and its effect on the mechanical properties of the treated hair fibers was limited, as seen in Figure 9. It is not fully explainable why chicoric acid is highly active in the polyphenols studied. We currently speculate that, due to a specific structure

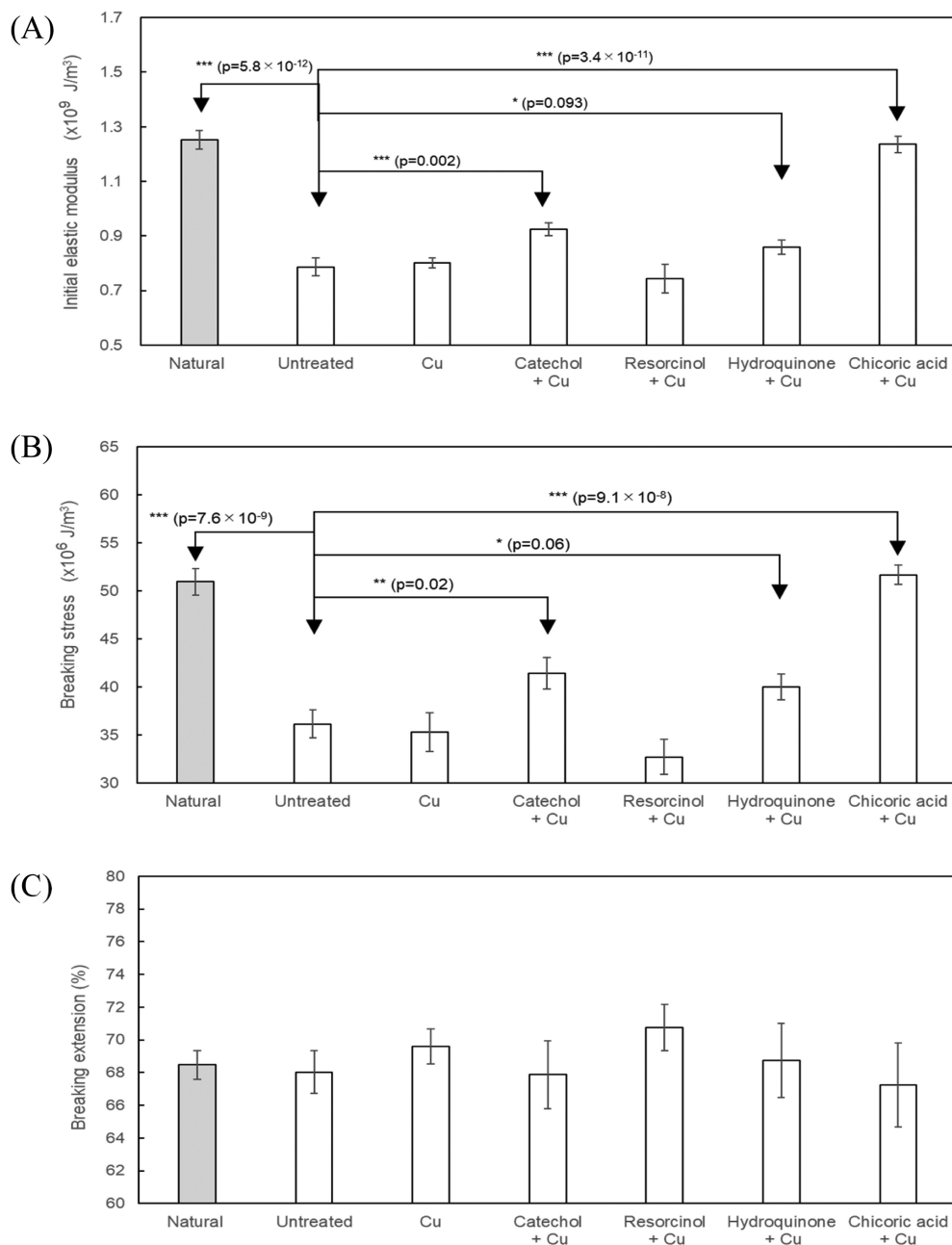


Figure 9. The initial elastic modulus (A), the breaking stress (B), and the breaking extension (C) of various hairs. The white bars in the bar charts are the bleached hair samples, untreated or treated, with various polyphenols (0.9 mM) and copper chlorophyllin (Cu, 4 ppm) at ambient temperature for 24h. See the experimental section for details. A t-test was used to compare between the two groups, which are shown with the arrows. The difference was assessed with two-side test. A total of 60 hair fibers (20 fibers from each of 3 persons) were tested. The asterisk indicates a significant difference ($p < 0.01$).

of the polyphenol which has two catechol-like moieties connected with a hydrophilic acid/ester backbone (Figure 1), the bidentate polyphenol acts efficiently on the cuticle keratin to cure the bleached hair, even in the low concentration. The previous report suggests that the large polyphenol may hardly penetrate the matrix (the cortex cellular region) through the thick layer of the cuticular cells of the hair.²³

CONCLUSION

First, the model proteins (lysozyme and keratin) were converted to higher molecular weight-proteins by treating with the polyphenol (catechol, hydroquinone and chicoric acid) in the presence of copper (II) chlorophyllin (4ppm). The amino acid analysis and the thiol group-quantification revealed that the polyphenol treatment induced the decrease in the contents of the lysine, histidine and cysteine residues of proteins. Second, the pale yellow, bleached, straight hair, which was made from black natural hair, was similarly treated with the polyphenols in the presence of the copper salt. The resulting light brown hair was examined for the amino acid analysis and the fluorescence characteristics to indicate that the amino acid residues were also decreased in a similar manner to the model proteins treated with polyphenol in the presence of the copper salts. It also appears that through treatment, especially with chicoric acid, the bleached hair was repaired at the levels comparable to that of the natural hair with the following characteristics: subdued cuticle scale lifting, small swelling degree, and large mechanical properties (the breaking stress and the initial elastic modulus). The results were explained by a formation of the quinone-crosslinking bond between the protein molecules as displayed schematically in Figure 7. The present treatment was conducted under mild conditions, and may be applied to hair-care technology for bleached hair damages.

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