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Dissimilar effect of perming and bleaching treatments on cuticles: Advanced hair damage model based on elution and oxidation of S100A3 protein

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Synopsis

Hair treatment chemicals induce sudden and severe hair damage. In this study, we examined cuticles from untreated, permed, and bleached hair that were mechanically discriminated by shaking in water. Both perming and bleaching treatments are prone to easily delaminate cuticles. Confocal microscopy revealed that the cuticles of permed hair were delaminated with larger pieces than untreated ones. On the other hand, the cuticles of bleached hair tend to fragment into small peptides. At the minimum concentration of thioglycolate required to elute S100A3 protein from the endocuticle into the reductive permanent waving lotion, enlarged delaminated cuticle fragments were observed. Although S100A3 is retained in bleached hair, S100A3 is irreversibly oxidized upon bleaching treatment. It is likely that the oxidative cleavage of disulfide bonds between cuticle-constituting proteins, including S100A3, results in the fragile property of cuticles. Here we present a more comprehensive model of hair damage based on a diverse mechanism of cuticle delamination.

INTRODUCTION

Although hair damage occurs gradually due to influences of weather and oxidants (i.e., environmental factors) and daily hair care (i.e., physical factors), topical application of

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hairdressing products (i.e., chemical factors) results in sudden and severe changes. Permanent waving and bleaching treatments during hair coloring processes are major causes of hair damage. In order to improve hair care products, it is essential to understand the mechanism of hair damage induced by these procedures.

The scale-like cuticle surrounds the hair cortex. Normally, cuticles are damaged prior to changes occurring within the cortex. We previously identified and characterized S100A3, a unique member with high cysteine content of the largest calcium-binding S100 protein family, characterized by tissue-specific distribution (1), in cuticles (2,3). Its soluble nature under reducing conditions is different from that of other hair proteinous components such as hair keratins and keratin-associating proteins. Ultrastructural localization of S100A3 in the inner part of cuticles (i.e., endocuticles) suggested its structural role in preserving the attachment of adjacent cuticles (4).

The release of S100A3 protein from the attachment site is suggested to be the most relevant event leading to hair damage. We previously proposed a hair damage model that implies the involvement of S100A3 in hair damaging processes (5). This model was based on the following four stages: (a) Newly emerging hair is characterized by a smooth-edged cuticle. (b) As a result of cracking of the edge of intact cuticles by normal grooming, the S100A3-rich layer in turn becomes the outermost layer. (c) S100A3, a soluble protein under non-reducing condition, is oxidized due to the cleavage of intermolecular disulfide bridges under environmental stresses such as UV radiation. (d) S100A3 is gradually released from hair during daily washing. It seems also possible that application of permanent waving lotion is able to elute S100A3 from natural hair within the second stage. However, it is still unknown how bleaching treatment induces hair damage without elution of S100A3.

In this study, we examined how chemical hair treatments affected cuticle delamination. Confocal microscopy revealed that permanent waving and bleaching treatments had dissimilar effects on the size of delaminated cuticles. In addition, 2-dimensional electrophoresis revealed an acidic shift of S100A3 due to irreversible modification of several residues out of the total of ten cysteines in oxidized hair. Based on these results, we present a comprehensive model of hair damage induced by chemical treatments.

MATERIALS AND METHODS

PERMANENT WAVING AND BLEACHING TREATMENTS

Hair was bleached by immersion in 1–10% hydrogen peroxide-ammonia solution (pH 9.5) for 1 hr at 37°C. Permanent waving treatment was done by immersing hair at 37°C in 0.1–3% ammonium thioglycolate solution containing 1% 2-aminoethanol and 0.45% ammonia for 1 hr, followed by neutralization by 7% sodium bromate for 15 min. Hair samples were collected from males of Japanese descent.

DELAMINATION OF CUTICLES

The mechanical isolation method for cuticles consisting of stirring hair in water (6) was modified with a small-scale delamination test as follows. One-cm hair fibers (200 mg) were stirred at 180 rpm using an R30 shaker (Taitec, Tokyo, Japan) with 10 ml of

distilled water containing 0.1% Tween 20 in a Falcon 50-ml conical tube (Becton Dickinson, Franklin Lakes, NJ) at 37°C for 20 hr. After passing through nylon mesh, the cuticle suspension was subjected to turbidity measurement at 600 nm (7). The amount of delaminated cuticle was estimated through OD_{600}, adopting the standard curve calibrated by the preweighed cuticle suspensions (Figure 1a).

SIZE MEASUREMENT OF CUTICLE FRAGMENTS

Delaminated cuticles were centrifuged at 14,000g for 10 min, and resuspended at 0.2% in 1% Tween 20 solution. Finally, the cuticle suspension was mixed with an equal volume of Vectorshield (Vector Laboratory, Burlingame, CA) on a slide and a cover glass put on. Utilizing an LSM510 fluorescent confocal microscope (Carl Zeiss AG, Göttingen, Germany) with a 488-nm argon laser light, we observed autofluorescence from delaminated cuticle fragments under the following setting parameters: pinhole: 200; detector gain: 900; ampl offset: –0.2; and ampl gain: 1.0. From the resultant confocal images, the averaged area of cuticle fragments was computed with NewQube gradational analysis software (Nexus, Tokyo, Japan).

ASSAY OF TOTAL AND S100A3 PROTEINS

Permanent-wave-lotion-immersed hair was concentrated by ultrafiltration after alkylation of thiol groups with iodoacetamide. Protein amounts were estimated using the Bradford assay (8). Human recombinant S100A3 protein was prepared as described previously (9,10). 1D-PAGE of S100A3 was performed using precast 4–12% Bis-Tris Nu-PAGE (7.8 × 6.3 × 0.1 cm) according to the supplier's manual (Invitrogen, Carlsbad, CA). The separation was started at 200 V for 35 min. S100A3 protein levels were quantified by Western blot analysis (5). Quantification was performed, using human recombinant S100A3 as a standard, by image-analyzing software (Scion, Frederick, MD).

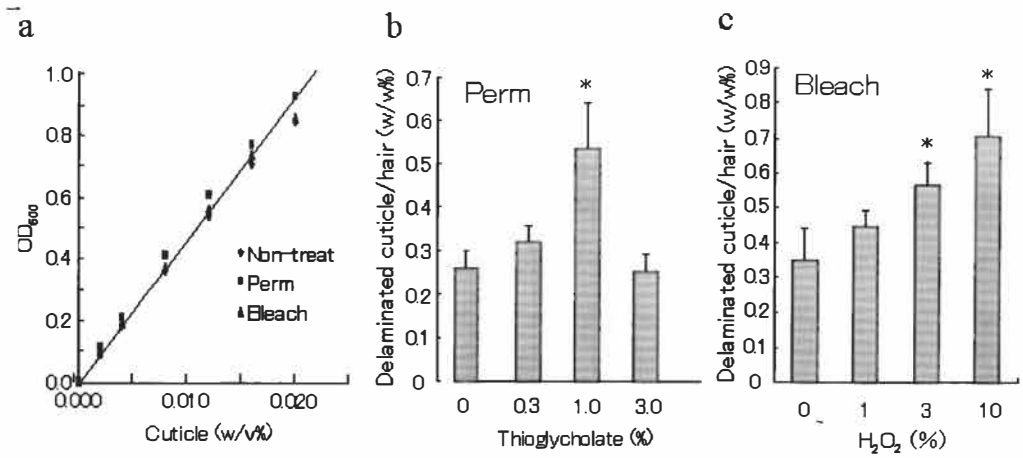

Figure 1. Increase of delaminated cuticles upon hair treatment chemicals. (a) Correlation between cuticle fragments and turbidity. Note the amounts of delaminated cuticle from permed (b) and bleached hair (c). Values represent the average of three independent experiments ± SD. Statistical analyses were performed by *t*-test of the values obtained without hair treatment chemicals. * $p < 0.05$.

2D GEL-ELECTROPHORESIS OF S100A3 IN DELAMINATED CUTICLES

Delaminated cuticles (1 mg) from bleached hair were extracted with 100 mM dithiothreitol containing 200 mM Tris-HCl buffer (pH 7.6). Extracts were concentrated by precipitation with trichloroacetic acid. The precipitate was applied to modified two-dimensional electrophoresis, as previously described (3). Isoelectric focusing was performed according to standard protocols using IPG strips with a narrow pH gradient of 3–5 (Sigma-Aldrich, St. Louis, MO). The second dimension was performed under the same conditions described above, using the Zoom type of Nu-PAGE gel at 7.8 × 6.3 × 0.1 cm. The mean density of spots and their proximal background zone on silver-stained 2D-PAGE gels were measured using image-analyzing software (Scion). Subtracted mean densities were used for proportion calculation.

RESULTS

At first, we examined whether the turbidity (OD_{600}) accurately represents the concentration of the cuticles in suspension. OD_{600} was linearly increased up to 1.0, depending on the amount of suspended cuticle (Figure 1a). No difference in OD_{600} was observed among non-treated, permed, and bleached hair samples. Consistent with previous reports (7), the cuticles of both permed and bleached hair were more easily delaminated compared to untreated ones (Figure 1b,c). The amount of delaminated cuticles from bleached hair was increased as a higher concentration of hydrogen peroxide was applied. In the case of permed hair, however, application of an excessive concentration (3%) of thioglycolate results in reduction of the delamination. This might be attributed to the lesser rubbing of the wavy hair with the water-stirring method.

Due to their very flat shape and their transparent nature, it is difficult to clearly identify delaminated cuticle fragments by conventional microscopy. In this study, we observed the autofluorescence of delaminated cuticles using confocal fluorescent microscopy (Figure 2a). Confocal images were processed using gradation analysis software. The transformed gradation image revealed that the average area of each cuticle fragment, delaminated from permed hair, was about twice as large as that of untreated hair (Figure 2b), whereas those of bleached hair were 30% smaller compared to the normal ones (Figure 2c). These results indicate that the physiological characteristics of cuticles from the bleached hair are distinct from those of the permed hair.

We previously detected S100A3 in the permanent waving lotion (5). We examined the correlation of S100A3 elution by perming treatment with the enlargement of cuticle delamination. Treatment of hair fiber at the minimum thioglycolate concentration required to elute S100A3 into permanent waving lotion ($\geq$0.3%, Figure 3) resulted in significant enlargement of the cuticles. Although application of higher thioglycolate concentration increased the amount of eluted S100A3 protein, the enlargement of delaminated cuticles reached a plateau at 1% concentration (Figure 2b). These results indicate that the loss of S100A3 protein, even in a low amount, results in cuticle delamination.

In contrast to permanent waving, bleaching did not result in a release of the S100A3 protein (data not shown). Nevertheless, cuticles of bleached hair were easily delaminated (Figure 1c) and fragmented into small pieces (Figure 2c). In this study, we performed 2D PAGE analysis of extracts of the cuticles delaminated from bleached hair. Although the

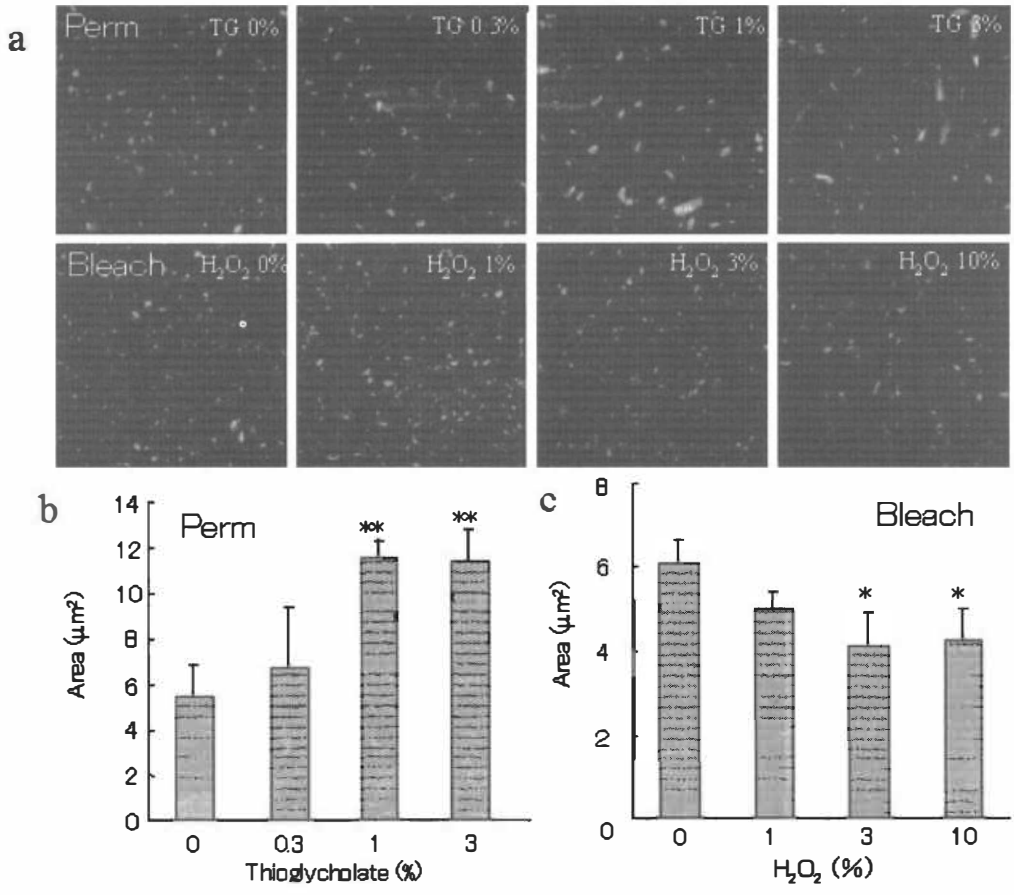

Figure 2. Effects of perming and bleaching on the size of delaminated cuticles. (a) Confocal microscopy of cuticles delaminated from permed and bleached hair. Cuticle suspensions were prepared from hair samples treated with various concentrations of thioglycolate and hydrogen peroxide (bottom row). Note the size of delaminated cuticles from permed (b) and bleached hair (c). The area of cuticle fragments was computed by gradational analysis of each confocal image. Values represent the average of three independent experiments ± SD. Statistical analyses were performed by *t*-test of the values obtained without hair treatment chemicals. * $p < 0.05$; ** $p < 0.01$.

precise identification of each isoelectric variant of S100A3 required further investigation, observed acidic shifts of the protein spots suggested that several cysteine residues in the S100A3 protein were converted into cysteic acid by oxidation with hydrogen peroxide. Our 2D PAGE analyses showed that S100A3 with a higher content of cysteic acid occupied a higher proportion in bleached hair treated with a higher concentration of hydrogen peroxide (Figure 4).

DISCUSSION

Various techniques, such as morphological observation of hair fiber using scanning electron microscopy and tensile measurement, have been employed to monitor hair damage. However, the critical differences between the perm and bleach inductive damaging mechanism(s) have never been studied. In this study, we report that the manner

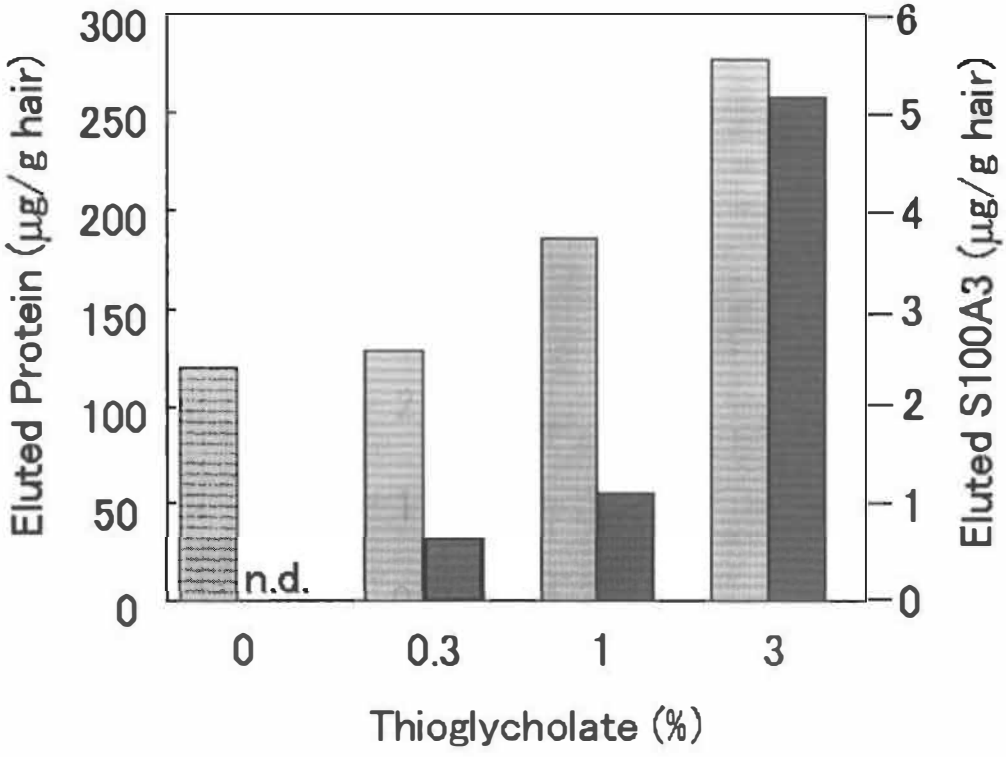

Figure 3. Total protein and S100A3 eluted from hair into permanent waving lotion. Total protein (gray box) and S100A3 (black box) levels were determined by Bradford assay and Western blot analysis, respectively. n.d., not detected.

of cuticle delamination processes largely depends on the experienced perming and bleaching treatments. Our results indicate that cuticles delaminated from permed hair are remarkably larger than those from untreated hair. Those from harshly bleached hair can, however, be smaller. Based on these findings, we present now a more precise model for chemically induced hair damage, which encompasses both S100A3 elution and oxidization during various hair treatment procedures (Figure 5).

We detected a close correlation between the concentration of S100A3 in the permanent waving lotion and the enlargement of the delaminated cuticles. We propose that reducing permanent waving lotion releases S100A3 from the inner part of the endocuticle (5) so that the cuticle becomes more easily delaminated. This study shows that the cuticles of permed hair tend to delaminate into large fragments. Interestingly, the loss of a low amount of S100A3 already damages the adhesion sites between the cuticles, and is sufficient to enlarge the cuticle fragments. This reinforces our postulation that S100A3 is a structural component linking adjacent cuticles.

Our previous hair damage model could not explain why in bleaching treatments induced hair damage occurs without loss of S100A3. Recent observation by transmission electron microscopy showed severe damage in the endocuticle upon bleaching treatment (11). This study has shown that several S100A3 spots were observed on 2D-PAGE without any chemical treatment, but the oxidized forms, shifted to acidic pI, increased dramatically by employing hydrogen peroxide. The acidic pI shift of S100A3 is observed within

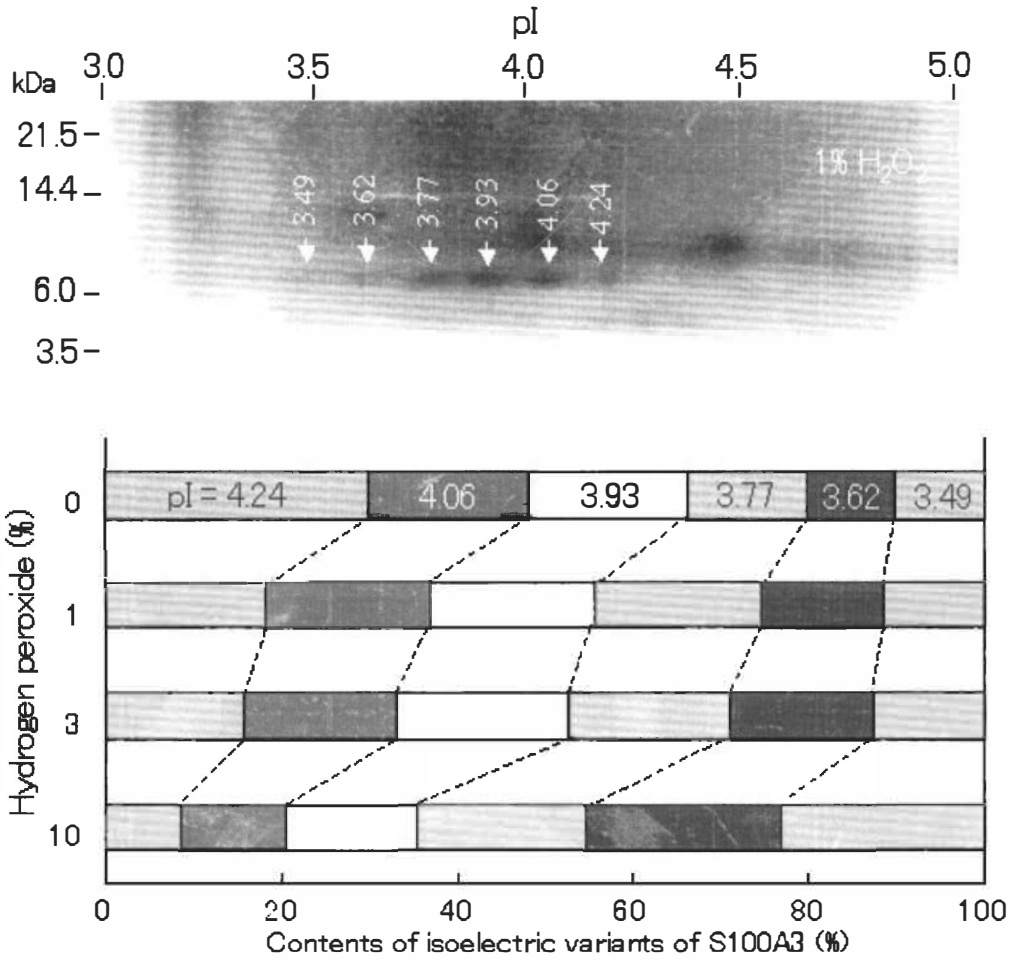

Figure 4. Proportion of various isoelectric variants of S100A3 in bleached hair upon 2D PAGE analysis. The upper panel shows the shift of S100A3 protein oxidized by 1% hydrogen peroxide to the lower pI. Each spot is at indicated pI. The lower panel shows the acidic pI shift of S100A3 spots in an H_2O_2 concentration-dependent manner.

the range of the concentration of hydrogen peroxide needed for cuticle delamination and its fragmentation into small peptides. We postulate that this molecular transformation represents whole cuticle oxidation, as most cuticle proteins, including S100A3, contain a higher portion of cysteines (12). Although most oxidized proteins were retained in cuticles even if some of the disulfide bridges were converted into cysteic acid, we postulate that the oxidized cuticles become more fragile due to the cleavage of disulfide bridges between cuticle proteins. A decrease in intermolecular crosslinks by bleaching treatment may result in the fragmentation of the cuticles into smaller peptides.

Our advanced model now suggests that the individual cuticles exhibit distinct physical properties, namely cuticle durability against mechanical stress, depending on the experienced hair treatment chemicals. This will enable us to improve our hair-care products suitable for each hair condition.

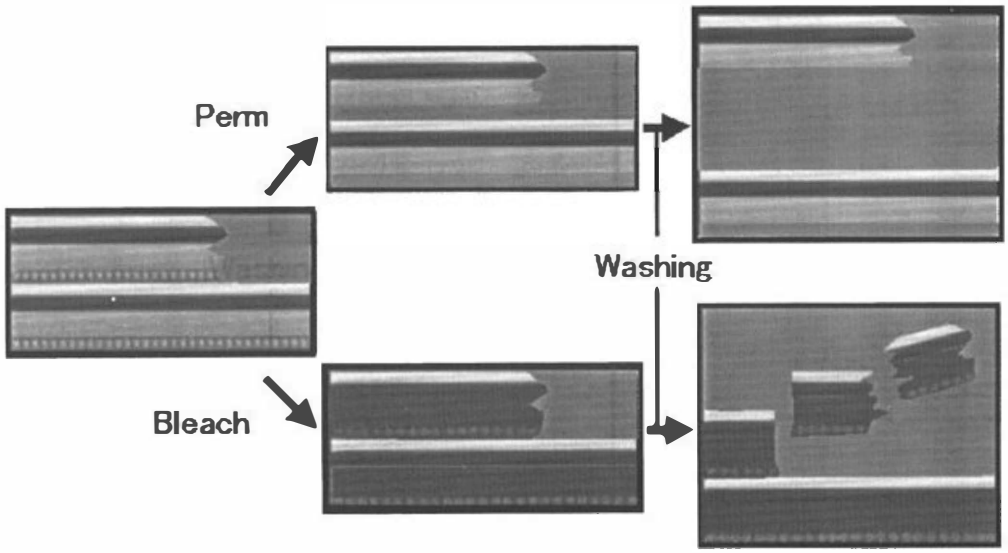

Figure 5. Advanced model encompasses hair damage mechanisms induced by both perming and bleaching treatments. This model starts from the "actual cuticle" chipped away at its edge in the second stage of our previous hair damage model (5).

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