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Chemical and photo-oxidative hair damage studied by dye diffusion and electrophoresis

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Synopsis

Microspectrophotometric and electrophoretic methods were used to characterize and quantify the effects of primary damage to hair from chemical and photochemical oxidative processes. The diffusion of molecules proceeding from the fiber surface to the center of untreated and modified (by chemical and photochemical oxidative processes) hair fibers was mapped by *fluorescence microscopy* and quantified by calculating diffusion coefficients of a fluorescent molecule. In addition, an *electrophoretic separation technique,* namely, SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), was used not only to substantiate the results obtained in the microfluorometric study, but also to show how the main classes of proteins of unaltered hair are modified by cosmetic chemical treatments, light exposure, and combinations of these two processes. UV microspectrophotometry is an alternate analytical method to evaluate photo-oxidative damage in hair, and supports the results obtained by microfluorometry.

INTRODUCTION

Natural weathering and grooming practices to improve appearance inflict irreversible damage to human hair. Grooming practices such as combing, blow drying, and brushing lead to mechanical damage, mainly to the surface of the hair fiber. On the other hand, chemical methods such as bleaching, perming, and photochemical oxidation result in chemical damage to the cuticle and cortex. The nature of this damage is in the form of cleaved chemical bonds, which are further oxidized to hydrophilic (acidic) functionalities. This alters the properties of the material, such as the extent of swelling and receptivity to other molecules that interact strongly with the acidic functionalities.

In undamaged hair, diffusion of dyes with a molecular weight of ~300–400 is relatively difficult and requires more than one hour to penetrate through the cuticular layers to reach the cortex. The molecular architecture within the cell structure restricts access to foreign molecules such as dye molecules. In chemically damaged fibers, on the other hand, cleavage of the disulfide bonds and further oxidation decrease the disulfide crosslink density in the matrix, and result in the formation of hydrophilic sulfonic acid groups. In some cases, damage can also result in cleavage of the peptide bonds and formation of carboxyl and amine groups, both of which are hydrophilic. The overall effect of such damage is an increase in swelling of the hair fiber (in the wet condition),

which in turn, results in improved access to dye molecules, and can be characterized by the diffusion coefficient. Therefore, the diffusion coefficient of a selected dye molecule (under specific dyeing conditions) can be used as a quantitative measure of the damage inflicted upon the fiber. Such methods are used in characterizing changes in the structure of synthetic fibers subjected to different processing methods (1).

This study investigates the effects of chemical and photochemical oxidative processes on (a) the microstructure of human hair and (b) the proteins from different histological components of hair. Using *microfluorometry,* oxidative damage is characterized and quantified by studying the changes in diffusion kinetics of the fluorochrome uranine into the hair shaft. The results of the UV radiation-induced photo-oxidative damage to the keratin structure are compared with those obtained in our earlier studies (2) with chemically bleached hair. Increased dye diffusion rates are indicative of changes in fiber morphology.

An *electrophoretic separation technique* was used to show how the main classes of proteins of unaltered hair are modified by cosmetic chemical treatments, light exposure, and combinations of these processes. The molecular weights of the extractable main classes of proteins of unaltered as well as chemically and photochemically altered hair were established. Decreases or increases in the amounts of extractable proteins relative to untreated hair suggest which proteins were modified by these chemical/photochemical treatments. Occurrence of new protein bands not observed in untreated hair is indicative of the treatment-induced breakdown of proteins, which were originally not extractable. On the other hand, the absence of protein bands in treated hair, which were observed in untreated hair, suggests further crosslinking of the protein network, which makes them less soluble and therefore less extractable.

EXPERIMENTAL

HAIR SAMPLES

Dark brown hair from DeMeo Brothers, New York, was used.

CHEMICAL OXIDATION

Bleaching was carried out by two different methods. In one method, hair was bleached for one and four hours with 6% alkaline hydrogen peroxide at room temperature (the pH was adjusted to 10.2 with ammonium hydroxide). The bleaching solution was freshly replaced every 30 minutes. In the other method, hair fibers were treated for 30 minutes with a bleach cream containing hydrogen peroxide and ammonium persulfate (pH 10.2). All samples were thoroughly rinsed, air-dried, and then placed in a desiccator for 24 hours.

PHOTOCHEMICAL OXIDATION—EXPOSURE TO SOLAR-SIMULATED UV RADIATION

Individual hair fibers were mounted in parallel on templates and exposed for a total of 100, 200, 300, 500, and 600 hours to alternating three-hour cycles of UV radiation and humidification as in a QUV accelerated weathering tester. This unit simulates the sunlight in the range of 290–400 nm, with an irradiance maximum at 340 nm. The

irradiance intensity factor was chosen to be 1.35, compared to 1.0 for regular sunlight. The energy density at the 340-nm wavelength was kept constant at 0.96 W/m^2. The total energy density in the wavelength range of 300–400 nm is 5.06 W/m^2.

MICROFLUOROMETRIC STUDY

Dyeing with uranine. The photo-oxidized hair fibers as well as the untreated controls were dyed in a 0.1% aqueous uranine solution (pH 7) at 50°C for 5.5 hours. The dyed fibers were rinsed thoroughly for several minutes in warm, distilled water, air-dried, and placed in a desiccator over P_2O_5 for 24 hours.

Sample preparation for microfluorometry. The dried, dyed fibers were embedded in Spurr's low viscosity resin, cured for 24 hours at 70°C, microtomed at 10-μm thickness, and viewed in a Leitz MPV 1.1 microspectrophotometer with the Ploem vertical illuminator in the narrow-band blue excitation beam.

Spectral scans were obtained on longitudinally viewed fibers, and the wavelength of maximum fluorescence (λ_m) was established at 540 nm. Spatial scans were made across the 10-μm-thick fiber cross sections at the established λ_m (540 nm).

Instrumental settings for spectral and cross-sectional scans

- Blue excitation: 450–490 nm; KP: 510 nm; LP: 515 nm
- Objective: 25× for spectral scans; 40× for cross-sectional scans
- Wavelength, λ_m: 540 nm for cross-sectional scans
- Measuring sensor: 20 × 30 μm^2 for spectral scans; 3.1 × 25.0 μm^2 for cross-sectional scans
- Accelerating voltage: 1.6 kv
- Scanning speed: 7.2 μm/s

Micrographs of the cross-sectional views were made with Kodak slide film at 160 ASA at 60-seconds exposure time, using a 25× objective, 1.6× collar, and a 10× ocular).

ELECTROPHORETIC STUDY

Hair samples/treatments. Hair samples with treatment sequences were:

- Untreated
- Untreated; 100 h/200 h/300 h UV
- Bleached 1 h/4 h with alkaline 6% H_2O_2
- Bleached 1 h /4 h as above; 100 h/300 h UV
- Bleached 1 h/4 h H_2O_2/300 h UV/bleached 1 h H_2O_2
- Permed 1×/3×
- Permed 1×/3× as above; 100 h/300 h UV
- Bleached/permed
- Bleached/permed/300 h UV

Extraction of hair proteins. From each of the above listed categories, 5 to 10 mg of 5-mm-long hair segments were immersed in an extraction buffer containing 0.05 M dithiothreitol (DTT) as reductant, 8 M urea as denaturing agent, and 0.05 M Tris (hydroxymethyl) amino-methane. The ratio of hair to extraction buffer was 1:100. The samples were extracted for 24 hours at ambient temperature, and finally sonificated for 30 minutes. The following reaction with DTT helps to open the keratin matrix to

facilitate the diffusion of solubilized protein out of the hair fiber. The reduction reaction is given as:

$$K\text{-}S\text{-}S\text{-}K + HS\text{-}(CH_2)_4\text{-}SH \rightleftarrows 2\ K\text{-}SH + (CH_2)_4\text{-}S\text{-}S\text{-}(CH_2)_4$$

where K = keratin.

Derivatization of thiols. Derivatization was done according to the equation given below with 20% iodoacetamide for 30 to 60 minutes while sonicating at ~40°C. The ratio of extraction buffer/derivatization solution was 10:1. The samples were then centrifuged, and the supernatant liquid (containing the extracted/derivatized proteins) was taken and placed into new vials for either freezing or instant use.

$$K\text{-}SH + I\text{-}CH_2\text{-}CONH_2 \rightarrow K\text{-}S\text{-}CH_2\text{-}CONH_2 + HI$$

where K = keratin.

SDS electrophoretic separation technique. SDS (sodium dodecyl sulfate)-PAGE (polyacrylamide gel electrophoresis) separates proteins exclusively according to their molecular weight. The proteins are loaded with the anionic detergent SDS, about 1.4 g SDS per 1 g protein, and therefore, build SDS-protein micelle complexes with approximately a constant net charge per mass unit. Therefore, all SDS-protein micelle complexes are highly negatively charged and rapidly migrate towards the anode, which leads to rapid separation. Therefore, it is important to note that *this separation is based primarily on molecular weight, because SDS protein micelle complexes have a similar characteristic net charge per mass unit.*

The extracted, denatured, and derivatized proteins, now unfolded into polypeptide chains, are entered into the wells of the gel alongside the protein standard of known molecular weight (up to ~200 kD). When the smallest protein has traveled nearly to the bottom of the gel, the electrophoretic run is stopped. The gel is removed from the chamber and then stained and destained, causing the stained gel to become lighter, but leaving a series of bands indicating the presence of separated proteins. The gel is then scanned into the computer for recording and enhancement of the digital image. The relative intensity of the bands in various lanes of the gel indicates the relative amount of each extracted protein. The molecular weights of the unknowns were established by comparing their relative electrophoretic mobility with those of the protein standard. The relative distance the protein travels down the gel is directly related to the log of its molecular weight. The greater the molecular weight, the smaller the relative electrophoretic mobility of the protein and the shorter the relative distance traveled down the gel, and vice versa.

Microcrofluorometric scans along the bands in the various lanes of the gel can be made to quantify the relative amount of each of the extracted proteins. Quantitative comparisons can be made.

RESULTS AND DISCUSSION

MICROFLUOROMETRY

Background. Dye diffusion rates in keratin are strongly affected by changes in fiber morphology (3). Therefore, dye diffusion rates are used to quantify damage to the hair fiber by oxidative processes. Oxidative processes are known to decrease the disulfide

crosslink density in the matrix and form highly hydrophilic sulfonic acid groups (3). This leads to an increase in swelling and increased dye diffusion rates. Therefore, the higher the diffusion rate of a molecule into the hair fiber, the greater the changes in fiber morphology, that is, the greater the damage to the fiber.

(a) Mapping of uranine diffusion in bleached hair. Our earlier studies involving chemical oxidation of hair fibers with hydrogen peroxide or a bleach cream containing ammonium persulfate had shown significant increases in the diffusion rates of uranine into the hair shaft, which is indicative of severe chemical and structural modifications resulting from the oxidative processes (2). In the present work, we have extended this method of using the diffusion of uranine as a measure of characterizing and quantifying morphological damage to photo-oxidatively degraded hair fibers.

(b) Mapping diffusion of uranine in photochemically oxidized hair. Uranine is an anionic fluorochrome, which produces a green fluorescence in a basic environment and a yellowish-green fluorescence in an acidic solution. The fluorescence emission spectrum of diffused uranine in a dyed/unaltered hair fiber is shown in Figure 1. From the emission spectrum, the wavelength of maximum fluorescence of uranine is established at λ_m = 540 nm, and the instrument is calibrated at that wavelength to carry out the profiling of the diffused uranine in the hair fiber cross section.

Representative fluorescence emission profiles of diffused uranine in untreated hair and hair fibers exposed to increasing cycles of UV radiation/humidification, accompanied by the corresponding micrographs, are shown in Figure 2a-c.

It can be clearly seen that in the untreated fibers dyed under identical conditions to those of hair fibers exposed to UV radiation/humidification cycling, diffusion of the dye molecule is restricted to the cuticula (Figure 2a). However, in the photo-oxidized samples, increased diffusion of the dyestuff into the fiber interior occurs with increased exposure time (Figure 2b,c).

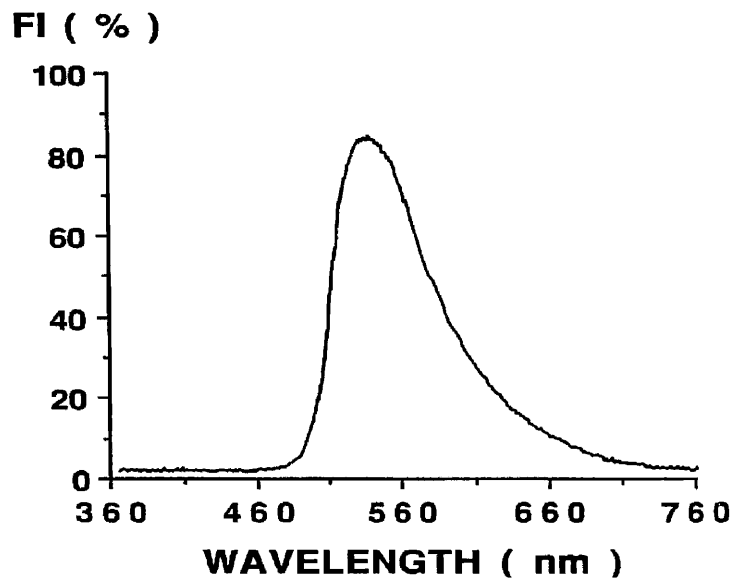

Figure 1. Typical fluorescence emission spectrum of uranine in an untreated hair fiber.

Calculating diffusion coefficients of uranine in untreated and photo-oxidized hair. The diffusion profiles are used to calculate the diffusion rates of uranine into photo-oxidatively damaged hair fibers. From the diffusion profiles, the fluorescence intensities, which are proportional to concentration, were sequentially read at normalized distances "r" from the center of the fiber of radius "a." The fluorescence intensity at the "saturated" fiber edge is read as well. Using the concentration ratios C/C_O for a specific, normalized distance "r" into the fiber with the radius "a," and for the fiber edge, an estimated diffusion coefficient was obtained from curves drawn by Carslaw and Jaeger (4) for Dt/a^2 by plotting C/C_O vs r/a.

This estimated diffusion coefficient from the Carslaw and Jaeger curves is then incorporated into an equation to calculate the more accurate diffusion coefficients of the dye molecule into the hair shaft. The equation used is a modification of the Crank (5) equation for cylindrical systems, using the roots of the Bessel functions of the first kind of the order zero (1).

$$\frac{C}{C_O} = 1 - 2\sum_{n=1}^{\infty} \exp\left(\frac{-Dt}{a^2} \cdot \beta_n^2\right) \cdot \frac{J_o(r\beta_n/a)}{\beta_n J_i(\beta_n)} \qquad (1)$$

The diffusion coefficient is calculated from equation (1) by iteration.

The calculated data are listed in Table I, which show significant changes in the diffusion coefficients of uranine in human hair fibers as a result of photochemical oxidation. For comparison, diffusion coefficients of chemically bleached (peroxide; bleach cream) hair fibers from our earlier work (2) are included.

The data in Table I are displayed in a graph in Figure 3. The graph and the table clearly show the increase in diffusion coefficients with the increase in the intensity of chemical and photo-oxidative processes. Clearly, the bleach cream containing ammonium persulfate is the most effective oxidative process, that is, it causes the greatest modification of the morphology of the hair fiber, resulting in the greatest increase in the dye diffusion coefficient. This is indicative of the highest level of oxidative damage. The four-hour peroxide treatment does not show any significant increase in diffusion rate compared to the shorter, one-hour, bleaching time. Apparently, most of the morphological change to

Table I
Diffusion Coefficients of Uranine in Untreated and Modified Hair Fibers

Hair sample	D ($m^2/s \times 10^{-15}$)
0 h peroxide	3.59 ± 0.67
1 h peroxide	9.78 ± 3.67
4 h peroxide	10.30 ± 0.87
0.5 h bleach cream	45.21 ± 28.66
0 h UV	4.34 ± 2.66
200 h UV/humidification cycling	6.47 ± 5.44
300 h UV/humidification cycling	8.62 ± 7.95
500 h UV/humidification cycling	18.18 ± 6.72
600 h UV/humidification cycling (right side)	23.16 ± 12.78
600 h UV cycling (left side)	20.39 ± 7.76

Sample size: ~10 hair fibers/category and ~5 diffusion coefficients/hair fiber.

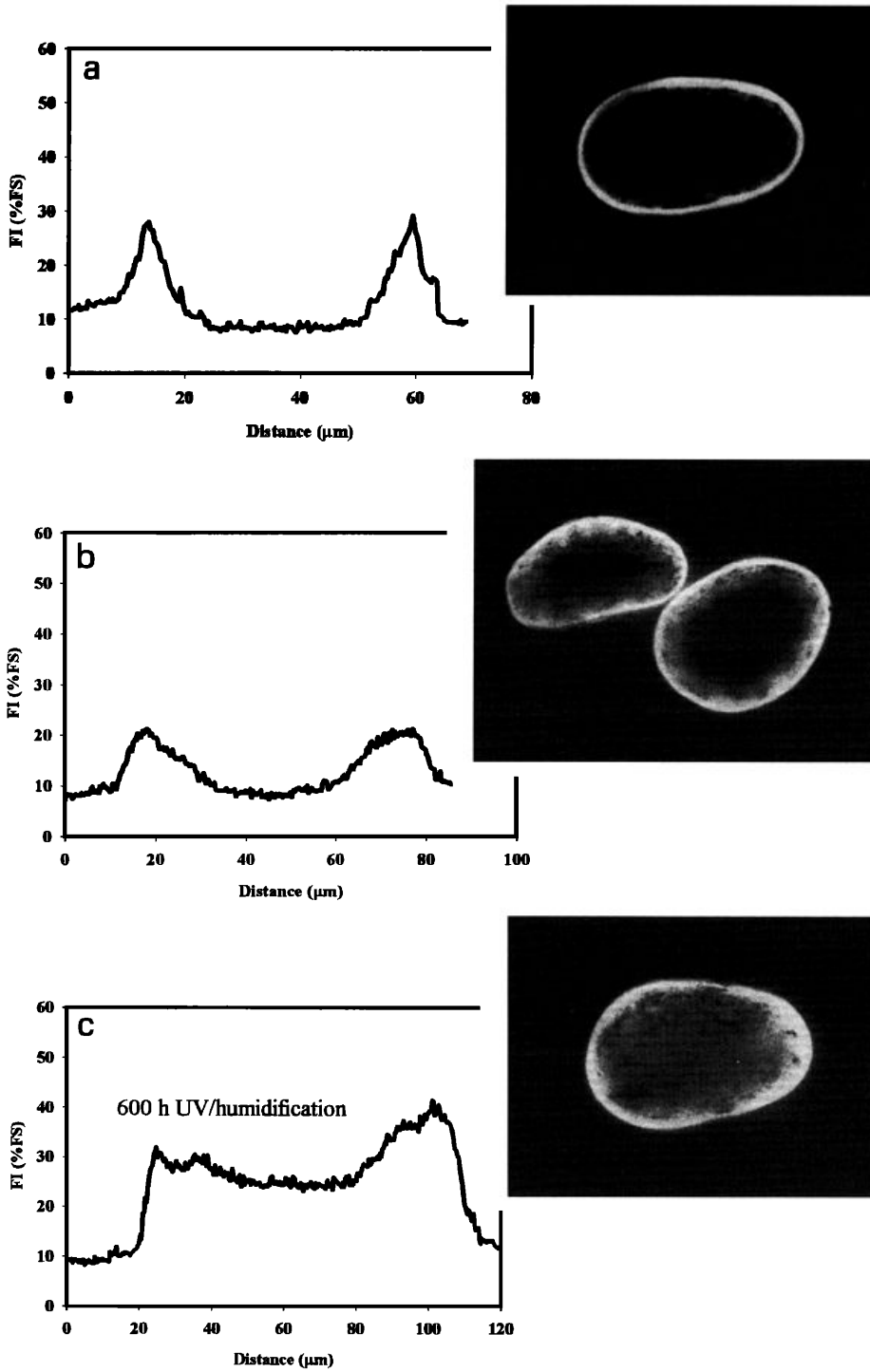

Figure 2. Micrographs and fluorescence emission profiles of diffused uranine (5.5 h) in (a) untreated hair and in hair fibers exposed to (b) 200 h and (c) 600 h of UV/humidification cycling.

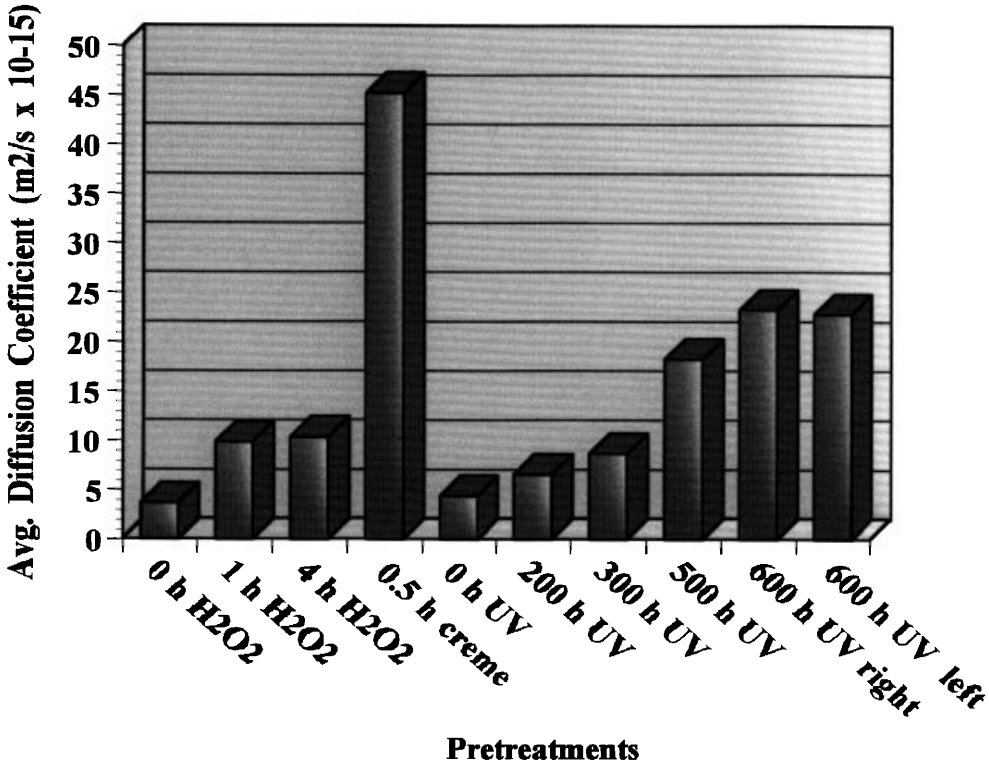

Figure 3. Effects of chemical and photochemical oxidative processes on diffusion kinetics of uranine in human hair.

the hair fiber has occurred during the short-term treatment, and little or no significant additional damage is inflicted, because of the loss of hydrogen peroxide by side reactions.

The newly obtained diffusion coefficient for the untreated hair fiber agrees well with the value obtained in the earlier work. The plot and table clearly show that the oxidative damage in hair fibers exposed to short-term (200 and 300 h) UV/humidification cycling is less than that of hair fibers bleached for one and four hours. However, hair fibers exposed to long-term (500 and 600 h) of UV/humidification cycling show greater oxidative damage than from both one and four hours of bleaching with peroxide. In general, long-term exposure to UV radiation results in damage greater than that observed for hair bleached with hydrogen peroxide, but less than that for hair bleached with hydrogen peroxide and ammonium persulfate.

It has to be pointed out, however, that each of the diffusion coefficients listed in Table I and shown in Figure 3 is an average value along the diffusion front of uranine into the hair shaft of several hair fibers. It is therefore important to look at the diffusion coefficients of uranine into an individual fiber because of the significant variation in diffusion coefficients within a given fiber. In the UV-exposed fibers, diffusion coefficients show a clear skin/core differentiation. As can be seen in Figure 4, the diffusion coefficient in the fiber periphery is significantly higher than that in the core of the fiber. This may be due to two reasons: (a) This gradient may be due to greater oxidative damage in the fiber periphery than in the fiber interior, which in turn results in greater fiber swelling

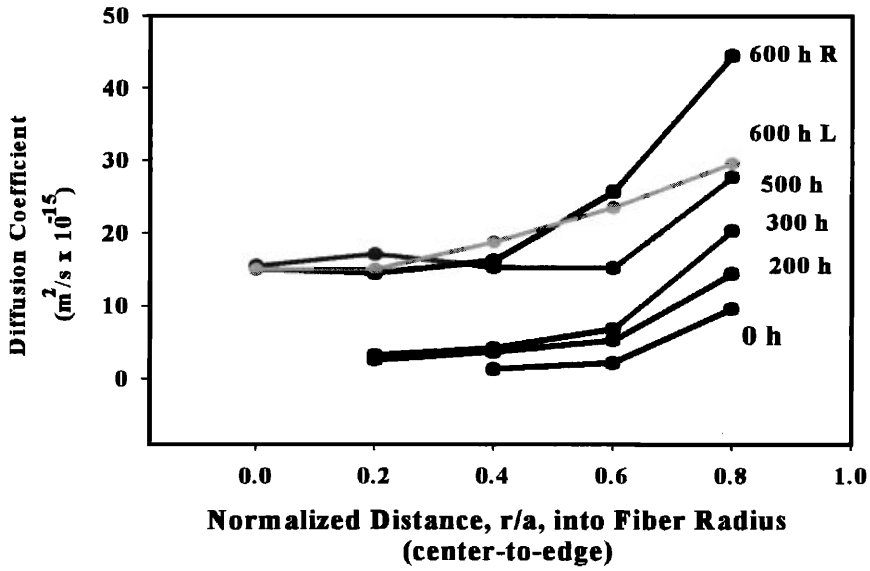

Figure 4. Radial distribution of the diffusion coefficients in a hair fiber.

and increased dye diffusion/uptake in the more severely oxidized/damaged peripheral region. (b) It may also be argued that besides increased oxidative damage in the fiber periphery, there is a concentration/location dependence, due to dye-strike on the surface and heavier dye uptake in the fiber periphery than in the fiber interior. Therefore, the diffusion coefficients may be concentration-dependent. The overall effect is likely to be a combination of the two effects.

The plots of the concentration ratio C/C_O as a function of location along the fiber radius (Figure 5) show a much more gradual diffusion gradient as we move from the edge into

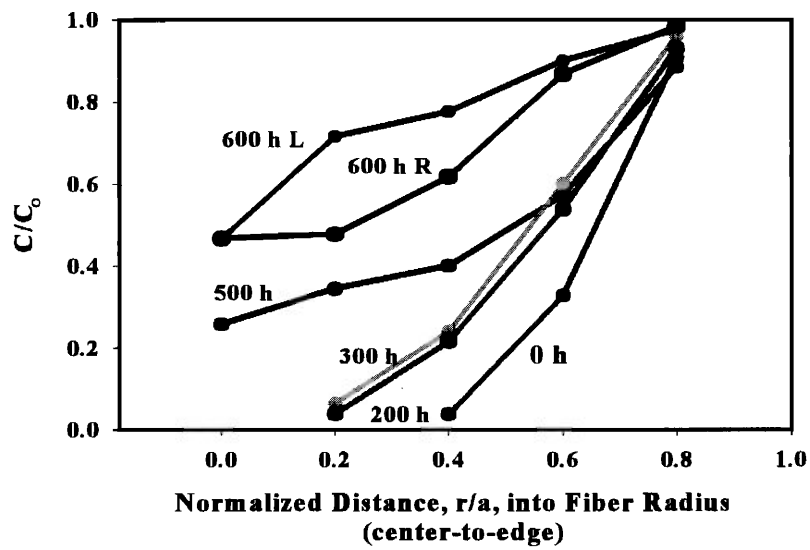

Figure 5. Dye concentration ratio as a function of location in the fiber radius.

the center of the hair fibers. These curves correlate well with the dye concentration profiles and the micrographs.

UV MICROSPECTROPHOTOMETRY

We have used UV microspectrophotometry to characterize photodamage in hair (6). Typical UV absorbance profiles at 340 nm of an untreated and UV-exposed fiber are shown in Figure 6a,b. The absorbance profile of the 300-hour photo-oxidized fiber shows that the photo-oxidized products are formed throughout the fiber cross section, with higher concentrations at the periphery. The profiles resemble the microfluorometric scans, suggesting that the photo-oxidized proteins are responsible for the increases in diffusion coefficients, thus supporting the microfluorometric approach adopted in this study.

ELECTROPHORESIS

Extractable proteins of unaltered and oxidized hair. The goal of electrophoretic separation was to determine oxidative damage to the main classes of proteins of unaltered hair modified by cosmetic chemical treatments, light exposure, and combinations of these processes. The molecular weights of the matrix proteins are in the range of 10 to 30 kDa, and those of the intermediate filament (microfibril) proteins are in the 40–55 kDa range (7).

First, this study investigated the extractable main classes of proteins of unaltered as well as chemically and photochemically altered hair. In the case of oxidized hair, decreases/increases in the amount of extractable proteins of a particular molecular weight, which are observed in untreated hair, suggest that these proteins were modified by chemical/photochemical treatments. The occurrence of new protein bands not observed in untreated hair is indicative of treatment-induced breakdown of proteins that had originally not been extractable. On the other hand, the absence of protein bands in treated hair, which were observed in untreated hair, suggests further crosslinking of the protein network, making it less soluble and therefore less extractable. Electrophoretic separation patterns are shown in Figures 7 and 8.

This work shows that perming and UV irradiation may crosslink and/or fuse the matrix and intermediate-filament proteins, turning them into insoluble and, therefore, less extractable high-molecular-weight proteins. Long-term bleaching with peroxide and the use of bleaching/perming combinations, on the other hand, appear to degrade the matrix, intermediate-filament, and high-molecular-weight proteins, predisposing them to accelerated solubilization and extraction. These conclusions are based on Figures 7 and 8.

Figure 7 clearly shows the effect of chemical and photochemical treatment on hair proteins. Lanes 1 and 10 show typical protein bands of the broad range standard. Lane 2 shows most of these protein bands as well, but at negligible concentrations. These are the typical protein bands of unaltered hair.

The extractable amount of the matrix and intermediate-filament proteins significantly decreases with increasing exposure to UV radiation (lane 3 = 100 h; lane 4 = 200 h; lane 5 = 300 h). In the case of the intermediate-filament proteins, extraction is completely

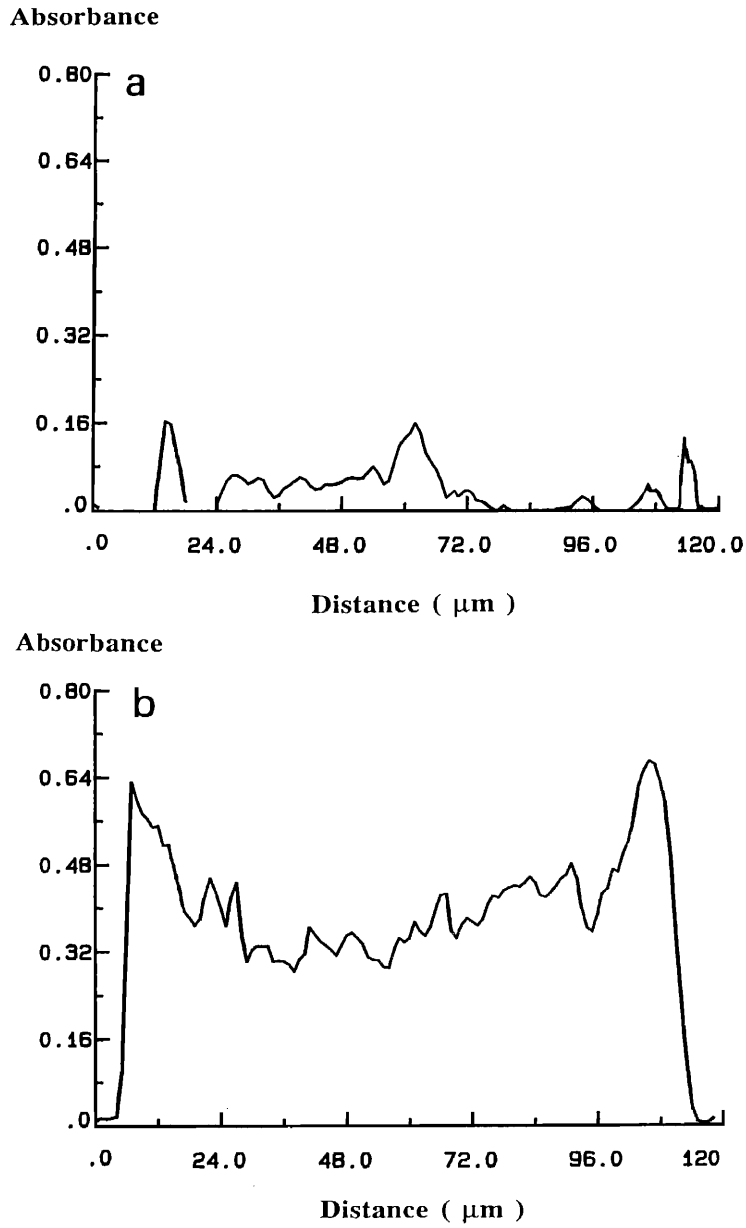

Figure 6. Typical cross-sectional scans across stabilizer-free hair fibers (a) before and (b) after UV exposure. (Scans were carried out at λ_m = 340 nm, the absorbance maximum of photodegradation products in hair.)

inhibited after long-term UV radiation (lane 5 = 300 h). However, a faint protein band develops after 300-h UV exposure in the region of *low* electrophoretic mobility, which indicates extraction of very small amounts of a *high-molecular-weight* protein. The molecular weight of this protein is more than 100 kDa. These features suggest that UV radiation may crosslink the matrix and intermediate-filament proteins, turn them into insoluble high-molecular-weight proteins, and inhibit their extraction. High-molecular-

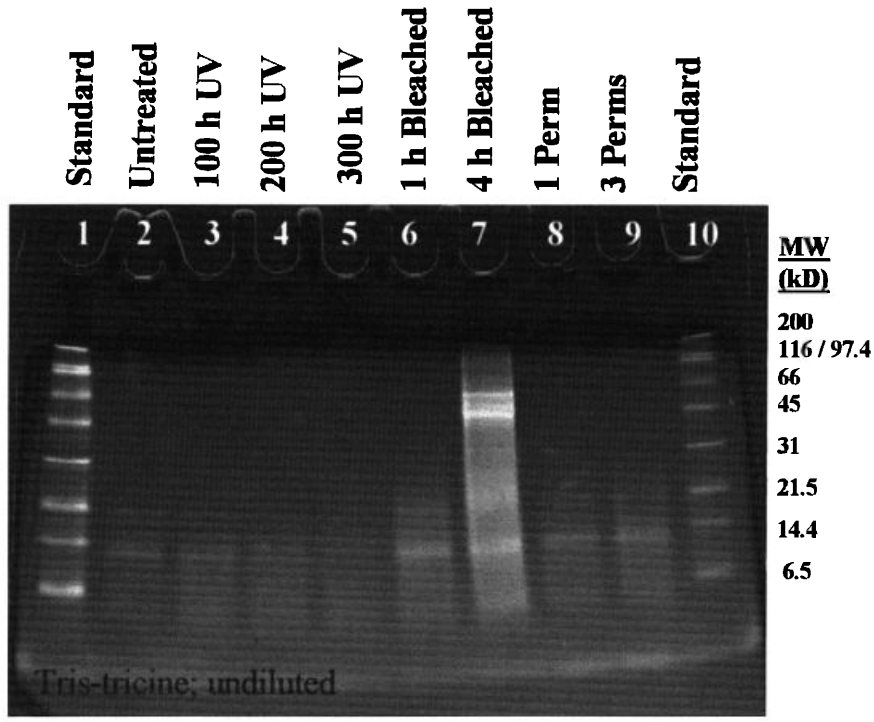

Figure 7. Electrophoretic separation of proteins of undamaged and chemically/photochemically treated hair.

weight proteins, on the other hand, may have been broken down by UV radiation into extractable components.

Significant increases in the extracted amounts of matrix, intermediate-filament, and high-molecular-weight proteins, especially from the four-hour-bleached hair sample (lane 7), suggest that bleaching with hydrogen peroxide (lanes 6 and 7) severely damages the hair proteins, preconditioning them for rapid solubilization and extraction. The once- and three-times-permed hair (lanes 8 and 9) shows elimination of the bands in the region of the intermediate-filament proteins, while the matrix proteins can still be extracted. This suggests that perming results in the solubilization of some matrix proteins in comparison to the unaltered hair (lane 2). The extracted amount is smaller than that observed in bleached hair.

Extractable proteins from hair exposed to combinations of chemical and photochemical treatments. Figure 8 shows the effects of combinations of chemical oxidation followed by photo-oxidative degradation of hair. Effects of the combination of bleaching followed by perming on hair proteins are investigated as well. Again, lanes 1 and 10 show the typical protein bands of the broad-range standard and lane 2 shows the extracted proteins of untreated hair.

Lane 3 shows easily extractable matrix, intermediate-filament, and high-molecular-weight proteins of the four-hour-bleached hair sample. Comparing lane 3 with lanes 4, 5 and 6, on the other hand, shows that the readily extractable intermediate-filament and high-molecular-weight proteins of the four-hour-bleached hair fibers (lane 3) become

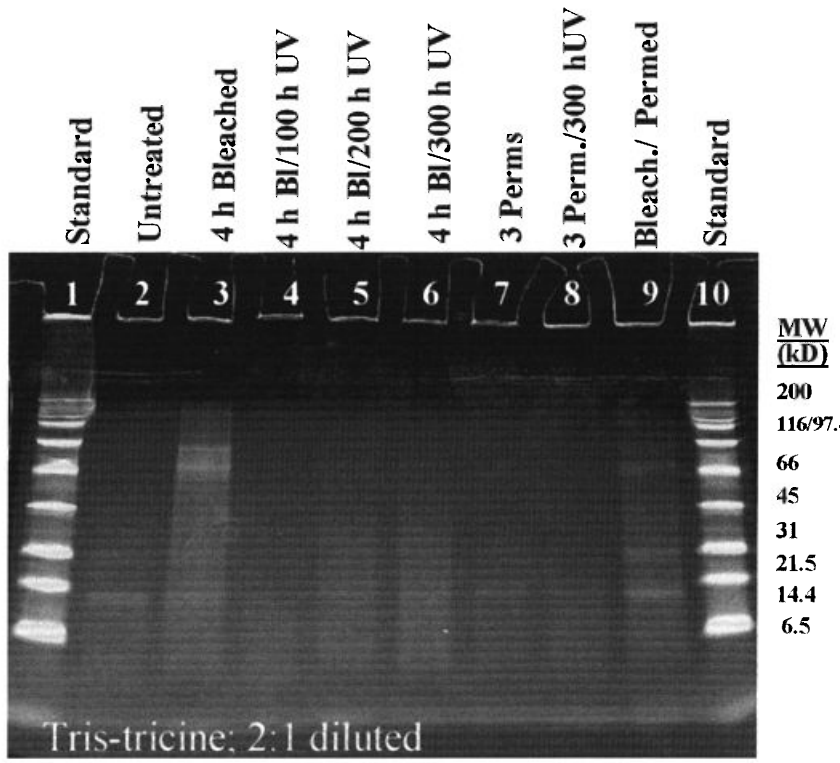

Figure 8. Electrophoretic separation of proteins of hair exposed to combinations of chemical and photochemical treatments.

much less extractable as a result of increasing UV exposure. This suggests UV-radiation-induced crosslinking of the intermediate-filament proteins, turning them into insoluble, less extractable, high-molecular-weight proteins.

Permed hair (lane 7) again shows a decrease in the amount of extracted intermediate-filament proteins, while the matrix proteins can still be extracted. Comparing lanes 7 and 8, it can be seen that the amount of extracted protein decreases even further in permed/UV-exposed fibers. This may suggest that not only perming, but also UV radiation, can turn the intermediate-filament proteins into less extractable, high-molecular-weight proteins.

Hair fibers exposed to a combination of short-term bleaching followed by perming (lane 9) show high amounts of extractable matrix, intermediate-filament, and high-molecular-weight proteins. This means that the combination of short-term bleaching and perming modifies the hair proteins in a similar way to the long-term four-hour bleaching of the hair sample. In both treatments, severe modifications of the hair proteins into soluble and extractable protein fragments had occurred. The effect of bleaching dominates.

A second study takes an additional step and looks at the effects of additional treatment combinations on the main classes of hair proteins:
(1) Large amounts of easily soluble/extractable matrix, intermediate-filament, and high-molecular-weight proteins had been formed in hair fibers exposed to a combination of treatments involving bleaching followed by perming. The question we want to answer

here is whether subsequent UV radiation will crosslink and/or fuse these soluble/extractable proteins and convert them into less soluble, and therefore, less extractable high-molecular-weight proteins.

(2) The readily extractable intermediate-filament and high-molecular-weight proteins of the four-hour-bleached hair fibers had become much less extractable as a result of possible crosslinking during increasing UV exposure. The question we want to answer in this study is whether subsequent peroxide bleaching of these originally bleached/UV-exposed fibers will again form readily soluble and thus easily extractable proteins, which had been obtained during the first bleaching sequence.

UV-radiation-induced crosslinking of hair proteins. Figure 9 shows the separation patterns from hair subjected to dual treatments. Lane 9 shows typical protein bands of the broad-range standard (reference). The readily extractable intermediate-filament and high-molecular-weight proteins of the four-hour-bleached hair fibers (lane 4) had become much less extractable as a result of increasing UV exposure (lane 5). It had been suggested that UV irradiation may crosslink and/or fuse matrix and intermediate-filament proteins, turning them into insoluble and less extractable high-molecular-weight proteins. It was theorized that a post-bleaching treatment with peroxide of these already bleached and UV-exposed fibers would degrade the UV-radiation-induced crosslinked and/or fused matrix and intermediate-filament proteins and turn them again

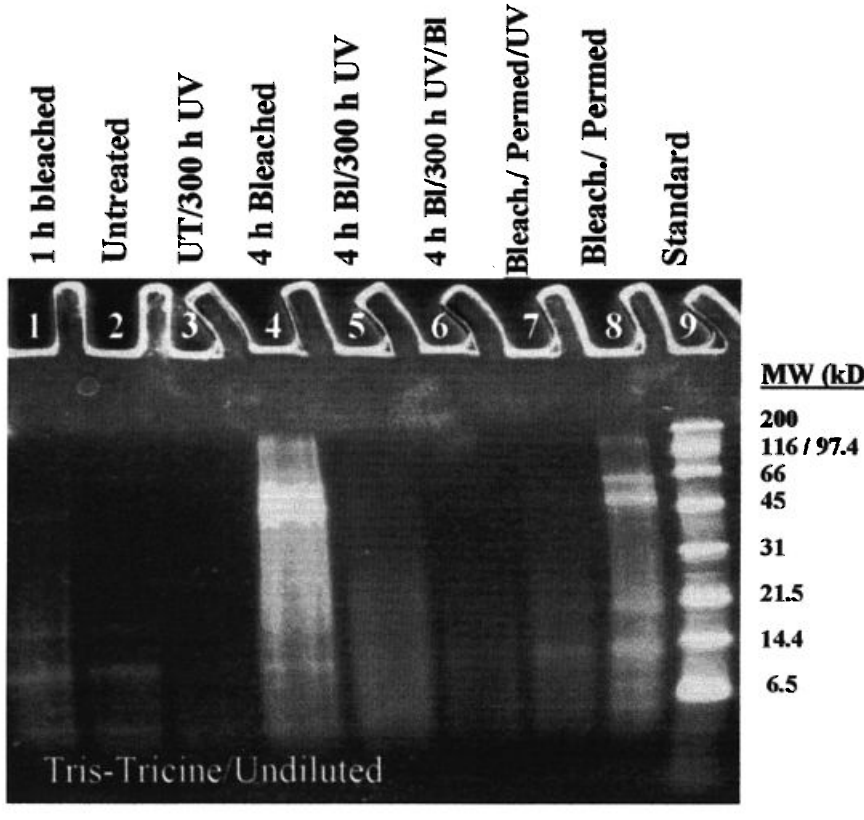

Figure 9. Electrophoretic separation of the main classes of proteins of hair exposed to combinations of chemical and photochemical treatments.

into easily soluble molecules. However, post-bleaching was not able to undo the UV-radiation-induced crosslinking and/or fusion of the proteins and degrade them into readily soluble/extractable molecules (lane 6).

Hair fibers exposed to a combination of short-term bleaching/perming show high amounts of extractable matrix, intermediate-filament, and high-molecular-weight proteins (lane 8). However, subsequent UV exposure results in a considerable decrease in extractable intermediate-filament and high-molecular-weight proteins (lane 7), suggesting UV radiation-induced crosslinking and/or fusion of the originally easily soluble/extractable proteins, which had been formed by bleaching/perming. Again, this seems to point to the possibility that UV exposure forms insoluble, and therefore less extractable, possibly high-molecular-weight, proteins by crosslinking. Embrittlement of the fiber supports this observation.

Earlier in-house SEM studies had shown that after severe DTT extraction of both the intermediate filament and matrix proteins, a completely collapsed, tube-like shell of the hair fiber remained, seemingly consisting mainly of the cuticular sheath. Under the conditions used in this electrophoretic study, no specific information is obtained about the cuticular proteins of this residual cuticular shell. It may be speculated that the proteins of the A-layer and exocuticle are resistant to extraction under the conditions used in this study. Prolonged extraction may be necessary to achieve their extraction. On the other hand, proteins of the weakly crosslinked endocuticular domains of the cuticle cell break down easily and are more extractable. However, their molecular weights may be comparable to those of the matrix proteins and, therefore, indistinguishable from the latter in the extracted materials.

CONCLUSIONS

The *microfluorometric study* has shown that chemical and photochemical oxidative processes severely damage hair proteins and, as a result, significantly alter fiber morphology. Modifications of fiber morphology due to oxidative processes are quantifiable by microfluorometry. There appears to be an initial resistance to photo-oxidative degradation. Short-term UV exposure appears to cause low levels of photodegradation, restricted to the peripheral region of the hair fiber. Comparing photochemical with chemical oxidation, short-term UV exposure results in slightly less damage than observed in hair fibers exposed to one and four hours of bleaching with hydrogen peroxide. However, long-term UV exposure results in more extensive oxidative damage than produced by chemical oxidation with hydrogen peroxide. The bleaching process involving the bleach cream containing ammonium persulfate, on the other hand, is more severe (the more effective oxidative process) than both photo-oxidative degradation and bleaching with alkaline hydrogen peroxide.

Electrophoretic separation techniques can be used to establish the damage done to hair proteins by chemical and photochemical treatments as well as combinations of both. This study suggests that UV irradiation and perming may crosslink and/or fuse matrix and intermediate-filament proteins, turning them into insoluble and less extractable high-molecular-weight proteins. Long-term bleaching with peroxide and the use of bleaching/perming combinations, on the other hand, appear to degrade the matrix,

intermediate-filament, and high-molecular-weight proteins, preconditioning them for accelerated solubilization and extraction.

These analytical methods are useful to quantitatively establish the level of morphological and chemical changes in the hair fiber caused by chemical and photochemical treatments as well as combinations of both. Such information will be helpful in selecting sunscreens, which are effective in preventing or retarding such damage.

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