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Spectrofluorescent characterization of changes in hair chemistry induced by environmental stresses

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Synopsis

Hair is frequently exposed to environmental stresses and chemical insults that result in damage to its internal structure and its outer cuticular components. Spectrofluorescence is a useful tool to monitor the health of biological tissues as it can measure the level of tryptophan (Trp), which is representative of protein integrity. In addition to Trp fluorescence, several other fluorophores are also present in hair and are believed to be attributed to kynurenenine, N-formylkynurenine, and 3-hydroxykynurenine, which are known metabolic and degradation products of Trp that are affected by environmental stresses normally experienced by hair. In this work, we were able to construct an endogenous fingerprint of fluorescent compounds present in hair by employing a range of excitation wavelengths from 270 nm to 450 nm with a resolution of 2 nm. As a result, we generated surface plots of fluorescence emission as a function of excitation and emission wavelengths (excitation-emission matrices). Thus, we were able to profile the levels of various structural molecules in hair before and after exposure to UV irradiation and thermal straightening irons as well as to chemical treatment such as bleaching and straightening.

INTRODUCTION

Human hair is constantly exposed to environmental insults that damage the fiber and reduce its biological integrity. In addition to natural stresses, such as UV irradiation, hair is often subjected to other chemical and physical treatments. For example, hair may be damaged by mechanical grooming procedures or thermal treatments carried out with hot irons. Chemicals treatments, such as permanent waving, hair straightening with relaxers, and bleaching of hair, are also very damaging to hair. Typically, permanent waving is accomplished by treating the hair first with a reducing agent (e.g., mercaptoethanol) to break disulfide bonds, then once hair is manipulated in the desired formation, it is treated with H_2O_2 in order to reform the disulfide links (1). Historically, hair straightening was achieved with lye relaxers (containing NaOH), which are very damaging to hair's morphological and chemical structure (2). More recently, slightly less basic reagents have been introduced as straightening agents; however, lye relaxers are still commonly employed. Hair bleaching, nowadays still a very common procedure, employs H_2O_2 used in conjunction with accelators (salts of persulfates), which results in hair surface damage and destruction of internal components of hair, including melanin and proteins (3,4). The subject of photodegradation of hair has recently received a great deal of attention in the

literature (5,6), while thermal degradation has been studied significantly less (7-9). Nevertheless, exposure of hair to either one of these insults results in internal protein damage (cortical) as well as detrimental effects to the outer cuticular components.

There are many biochemical techniques available, which allow for specific protein determinations, thereby permitting one to monitor the quantity of protein present in hair so that comparisons can be made among population samples. Unfortunately, these techniques require the destruction of the hair fiber and subsequent, time-consuming procedures and analyses. Measuring the fluorescence of biological tissues with an external fiber optic probe is frequently utilized due to its non-invasive nature and ease of use. Typically, Trp is monitored due to its intrinsic fluorescence and because it serves as an indicator of overall protein damage. Such studies were carried out to monitor Trp fluorescence and to investigate the presence of other fluorophores in hair (7,10–12). In the current study, we monitor the damaging effects of environmental and chemical insults to hair by employing steady-state fluorescence to generate excitation-emission matrices. The matrices provide full fluorophore characterization of the biological substrate under investigation.

EXPERIMENTAL

MATERIALS

In order to observe differences in hair damage resulting from hair pigmentation, studies were carried out with European dark brown (International Hair Importers & Products, Glendale, NY) and Piedmont hair (DeMeo Brothers, Passaic, NJ). Hair tresses were prepared by gluing 2 g of fibers to a 1.5-in. × 1.5-in. plexiglass tab with Duco Cement. The resulting dimensions of all hair tresses were 6.5 inches in length and 1.25 inches in width. Hair tresses were precleaned with a 3% ammonium lauryl sulfate solution and rinsed thoroughly prior to use in the experiments.

Hair relaxer treatments were carried out using Revlon Realistic Creme Relaxer (Colamer U.S.A., Inc., Dist.; New York, NY), which contains: water, petrolatum, parrafinum liquidum, cetearyl alcohol, propylene glycol, sodium hydroxide, polysorbate 60, cetyl alcohol, leneth-15, PEG-60 lanolin, potassium cocoyl hydrolyzed collagen, PEG-150 stearate, steareth-20, and fragrance.

Hair was bleached using Clairol Professional BW 2 (Clairol, Stamford, CT) bleaching powder containing potassium persulfate, ammonium persulfate, sodium metasilicate, sodium stearate, silica, hydrated silica, hydroxypropyl methylcellulose, aluminum distearate, sodium lauryl sulfate, disodium EDTA, and sorbitol. The bleaching powder (120 g) was mixed with 147 mL of Clairoxide 20 volume (Clairol, Stamford, CT) developer according to the manufacturers instructions. The developer contained water, H2O2, and phosphoric acid.

INSTRUMENTATION

Fluorescence measurements were carried out on a steady-state spectrofluorometer (FluoroMax-4) manufactured by Horiba Jobin Yvon (Edison, NJ) equipped with a bifurcated fiber optic probe. The fast and accurate scanning capability of the instrument (80 nm/s)

allows for collection of numerous spectra for one sample position. Excitation-emission spectra are constructed by measuring fluorescence emission at various excitation wavelengths—from 270 nm to 450 nm in increments of 2 nm. Emission spectra were collected starting 15 nm higher than the excitation wavelength to generate a total emission scan of 200 nm. Therefore, if the excitation wavelength was set at 270 nm, the emission spectrum would be collected from 285–485 nm. Average values represent measurements from two hair tresses in which case readings were obtained 1 in. from the top of the tress. In some cases, such as photo- and thermal degradation studies, readings were obtained 1 in. from the top of the tress (unexposed area) and 2 in. further down the tress in the exposed region.

Thermal exposure was conducted for 1 min. of continuous treatment in the middle of the tress using a Big Chi (Model GF1533D) flat iron marketed and sold by Farouk Systems Group (Houston, TX). It is a variable temperature controlled iron capable of reaching a temperature of 200 °C.

Hair was exposed to UV radiation in a Q-Sun weathering chamber (Q-Sun Xenon Test Chamber; Model Xe-1-B; Q-Panel Lab Products, Cleveland, OH) containing a full spectrum 1800 xenon arc lamp with irradiance set at 0.35 W/m2 at 340 nm. The total exposure time was 96 hours at a temperature of 40 °C. Hair was mounted in frames constructed of black cardboard shielding the top and bottom portions of the hair tress and leaving only the middle portion exposed to UV light.

RESULTS AND DISCUSSION

Previous studies of the intrinsic fluorescence of hair yielded information about the effects of chromophores on the fluorescent behavior of Trp and its metabolic/degradation products: Kynurenenine, N-formylkynurenine, and 3-hydroxykynurenine (12). The effects of melanin on the fluorescence of these molecules can be monitored by comparison of highly pigmented dark brown hair with non-pigmented Piedmont hair or white hair. In the current investigation we utilized Piedmont hair, which contains higher levels of yellow pigmentation than pure white hair. Gray hair may also contain yellowish coloration, presumably present due to exposure to UV radiation, which is believed to be attributed to higher levels of 3-hydroxykynurenine (11). In general, hair containing greater quantities of melanin has much lower emission characteristics, most likely because more light absorbed by melanin will result in less light available to interact with Trp and other fluorophores. Further, Trp fluorescence was found to be highly dependent on the moisture content of hair, with greater Trp fluorescence occuring at higher levels of hydration (12). Trp fluorescence is known to be extremely sensitive to its immediate environment, and may increase or decrease depending on the mobility of the Trp residues. One may even observe an increase in Trp fluorescence when hair is subjected to damaging treatments such as permanent waving due to the cleavage of disulfide bonds. Although one may expect less Trp to be present after such a damaging treatment, such an effect may be explained by an increase in the mobility of Trp residues in the protein in the absence of disulfide bond (13).

BLEACHING OF HAIR

In many cultures, hair bleaching is a widespread procedure that is employed to lighten the color of pigmented hair. It is especially popular for the formation of highlights—the

artistic blending and shading of hair to produce an overall configuration on the head containing the originally colored hair fibers with fibers of various degrees of lightening. The principal intention of hair bleaching is the destruction of melanin granules by oxidation through the action of H_2O_2 and persulfate salts.

Dark brown and Piedmont hair were bleached for 30 min. with a commercial bleaching formulation. For both cases, photographs of virgin and bleached hair are provided in Figure 1. While dark brown hair still contains some pigmentation after the bleaching process, Piedmont hair (yellow hue in the virgin state) becomes very white after treatment. Excitation-emission matrices for virgin and bleached dark brown hair are provided in Figure 2. The most apparent difference between the two spectra is the large increase (as a result of bleaching) of the fluorescence band corresponding to the principal kynurenine peak (I_{kyn}), which occurs at λ_{ex}=366 nm, λ_{em}=433 nm. There is also a large fluorescence increase observed at the extreme of the spectrum corresponding to λ_{ex}=450 nm, λ_{em}=509 nm, which we refer to as I_{509} throughout the text. Trp fluorescence (I_{Trp}), corresponding to λ_{ex}=290 nm, λ_{em}=343 nm, appears to decrease as a result of bleaching; however, this is due to a difference in scale of the two spectra. In fact, Trp fluorescence increases as a result of bleaching, probably because less melanin is available to absorb light.

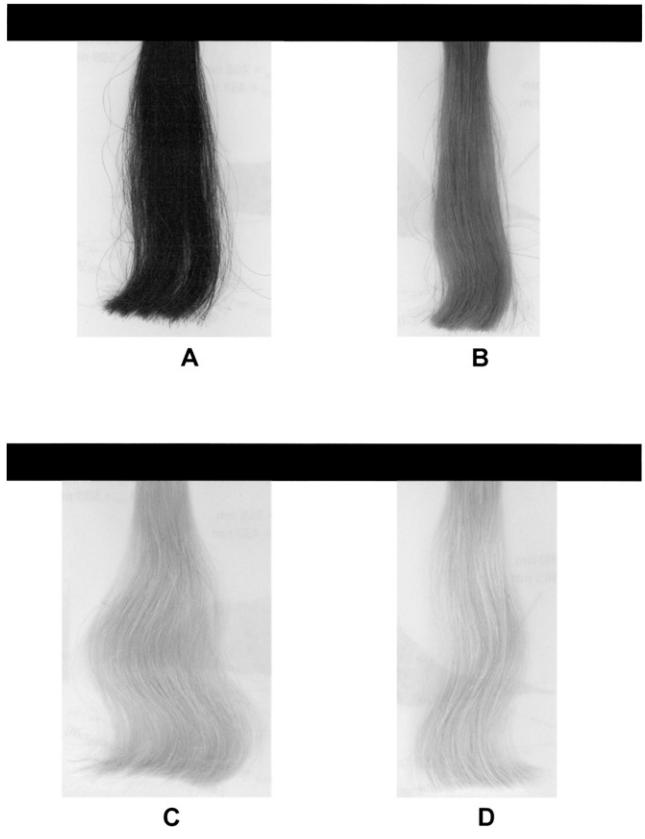

Figure 1. Photographs of (A) virgin dark brown hair, (B) bleached dark brown hair, (C) virgin Piedmont hair, and (D) bleached Piedmont hair.

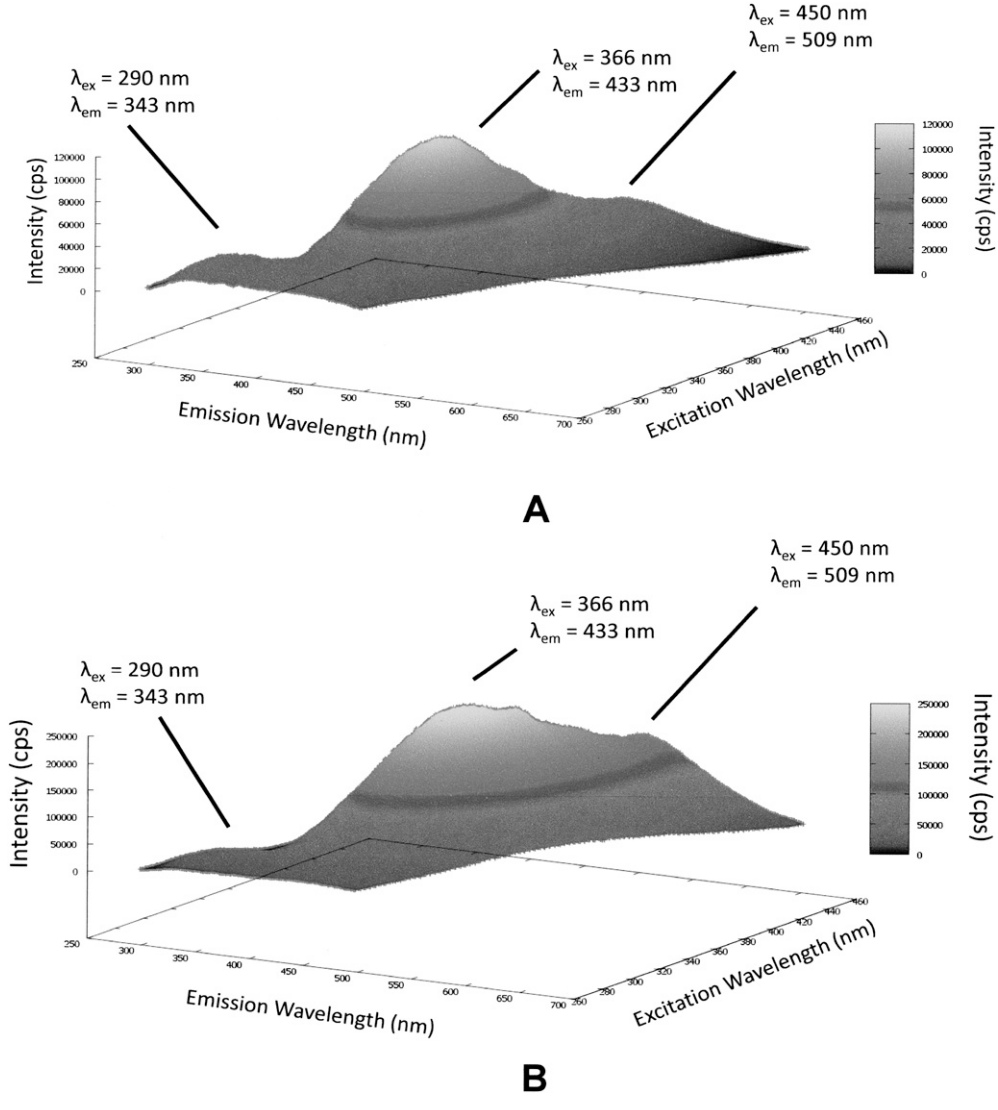

Figure 2. Excitation-emission matrices of (A) virgin and (B) bleached dark brown hair.

The corresponding spectra for virgin and bleached Piedmont hair are provided in Figure 3. A numerical summary of these data is provided in Table I. Here, we clearly see an increase in Trp fluorescence as a result of bleaching, both in the case of dark brown and Piedmont hair. We also see a large increase in the fluorescence of the principal kynurenine peak. The fluorescence signal of Trp increases only slightly for dark brown hair as compared to an almost two-fold increase in Piedmont hair. The principal kynurenine peak, on the other hand, more than doubles for dark brown hair and increases by one-half for Piedmont hair. The peak intensity at the extreme of the spectra remains the same for Piedmont hair. It is interesting to examine the changes in the ratio of I_{Trp}/I_{Kyn} and I_{509}/I_{Kyn}. Bleaching results in a decrease in I_{Trp}/I_{Kyn} for dark brown hair and an increase in this same ratio for Piedmont hair. Curiously, the ratio of the peak at the longest excitation

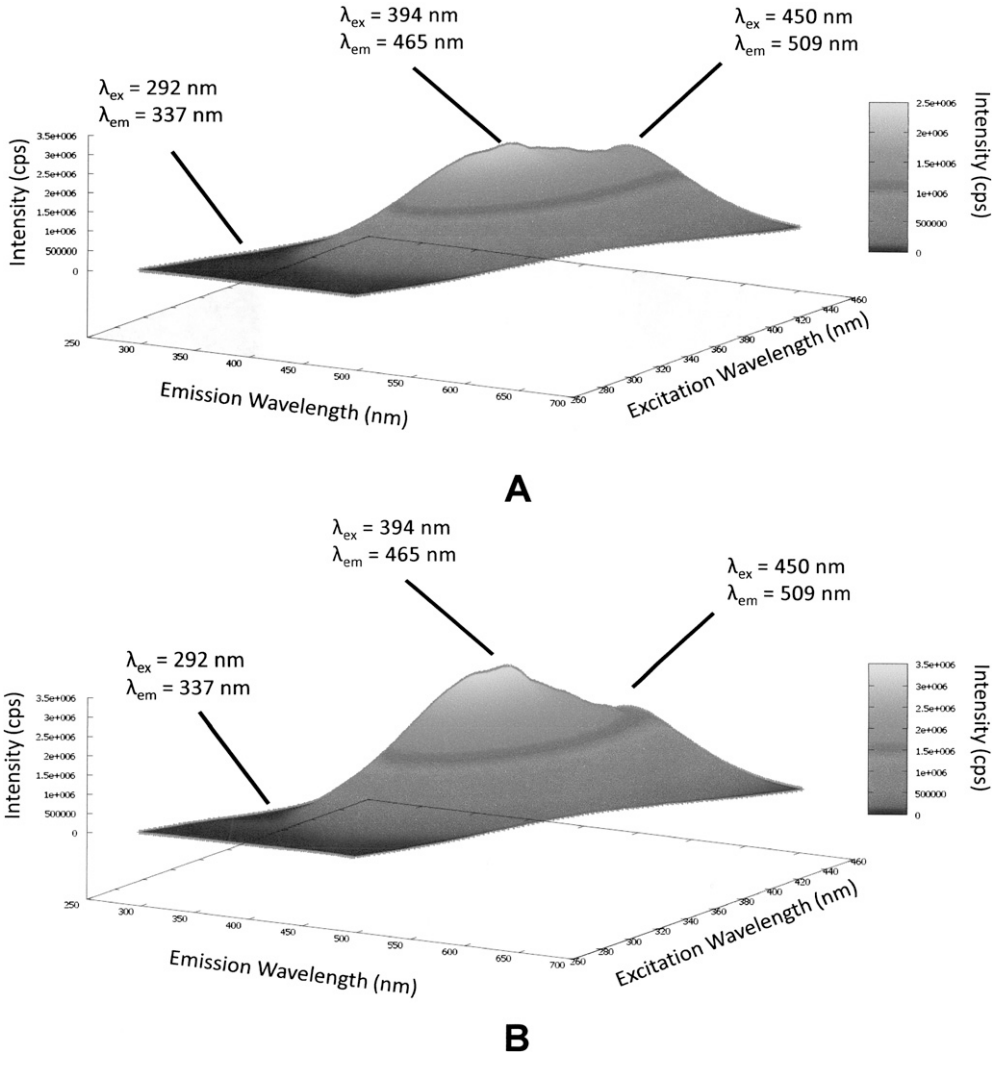

Figure 3. Excitation-emission matrices of (A) virgin and (B) bleached Piedmont hair.

band to the kynurenine peak, I_{509}/I_{Kyn}, decreases as a result of bleaching in Piedmont hair, even though the peak intensity increases. The decrease in this ratio parallels the loss of yellow pigmentation in Piedmont hair, something we also observed in photo-damaged Piedmont hair (see section below). In the case of dark brown hair, I_{509} increases dramatically resulting in a very large increase in I_{509}/I_{Kyn}. Some of the fluorescence increase at I_{509} could be due to the formation of degradation products; however, the large increase can most likely be attributed to decreased quantities of melanin.

Since bleaching changes the pigmentation characteristics of the fiber, it will also have profound effects on the fiber's fluorescence. In the case of dark brown hair, a decrease in the quantity of Trp relative to the kynurenines could indicate that any one (or a combination) of the following processes occur: Trp is degraded, kynurenines are formed as a result of Trp degradation, or melanin impedes kynurenine fluorescence more than Trp fluorescence. In

Table I
Peak Intensity Values and Pertinent Peak Ratios for Dark Brown and Piedmont Hair in the Virgin and Bleached States

	I_{Trp}	I_{Kyn}	I_{509}	I_{Trp}/I_{Kyn}	I_{509}/I_{Kyn}
Dark brown	27,300 ± 1,838	104,650 ± 7566	26,250 ± 212	0.261 ± 0.001	0.252 ± 0.020
Dark brown-bleached	31,000 ± 707	222,000 ± 22,627	137,500 ± 3,536	0.141 ± 0.017	0.622 ± 0.047
Piedmont	47,400 ± 2121	2,135,000 ± 134,350	1,540,000 ± 169,705	0.022 ± 0.000	0.720 ± 0.034
Piedmont-bleached	85,150 ± 2899	3,205,000 ± 49,497	1,585,000 ± 106,066	0.026 ± 0.000	0.494 ± 0.025

Piedmont hair the Trp signal increases as a result of bleaching. It is important to point out that Piedmont hair loses almost all of its pigmentation upon bleaching, especially its yellow hue, which more than likely comes from kynurenine (11,12,14).

HAIR STRAIGHTENING

Commonly employed by individuals whose hair can be characterized as very tightly curled (e.g. African type hair) or frizzy, hair straightening formulations (chemical relaxers) rely on the activity of strongly basic formulations which result in chemical and morphological changes in the hair shaft. Traditionally, NaOH based (lye) relaxers were employed to carry out such a task, resulting in a great deal of damage to the fiber including cleavage of disulfide bonds, changes in protein conformation (α-helix to β-sheet transitions), and the formation of lanthionine crosslinks. More recently, lower pH chemical relaxers have been introduced into the market place; however, they still damage the fiber. In the present study, we examine the effects of hair straightening with a lye relaxer on hair fluorescence.

The peak intensities and ratios for chemically relaxed hair are provided in Table II. As a result of relaxer treatment, Trp fluorescence decreases for both dark brown and Piedmont hair (compared to untreated readings in Table I). In contrast, the signal for I_{Kyn} is essentially the same as for untreated hair in both (dark brown and Piedmont) cases. Although Trp degradation to kynurenines may occur—which would lead one to expect an increase in I_{Kyn}—the kynurenines themselves may be degraded by the relaxer treatment. In the case of the peak at the highest excitation wavelength employed, I_{509}, relaxer treatment results in a large decrease in peak intensity for Piedmont hair and no change for dark brown hair. This effect may be more pronounced for Piedmont hair since its fluorescence in this region is much more discernible. The peak ratios are also provided in Table II, which correspond with the peak intensity observations.

THERMAL DEGRADATION OF HAIR

Frequently, hair is exposed to thermal treatments to provide desired hair set or style. In previous studies, we found that hair experiences surface (cuticular) and internal (cortical) damage as a result of thermal treatment (7). Hair also undergoes color changes upon exposure to heat. This is clearly evident in the photograph of Piedmont hair shown in Figure 4,

Table II
Peak Intensity Values and Pertinent Peak Ratios for Dark Brown and Piedmont Hair Exposed to Chemical Relaxer Treatment

	I_{Trp}	I_{Kyn}	I_{509}	I_{Trp}/I_{Kyn}	I_{509}/I_{Kyn}
Dark brown-relaxer	19,150 ± 636	108,000 ± 2,828	26,650 ± 1061	0.177 ± 0.001	0.247 ± 0.003
Piedmont-relaxer	26,150 ± 1,485	2,155,000 ± 35,355	728,700 ± 28,284	0.012 ± 0.000	0.672 ± 0.002

where one can visually observe the region of the tress where hot iron treatment was administered, resulting in the formation of a dark yellow hue. In the case dark brown hair, we do not observe a visually significant color change, probably because it is masked by the absorption of melanin. Thermal treatment was administered for 1 min. of continuous treatment. For this particular hot iron application, such a length of treatment might be considered extensive. However, this time-scale was meant to be representative of cumulative treatments (i.e. a series of short treatment protocols) providing an overall equivalent treatment time to the continous treatment. In fact, previous studies show that cumulative treatment is actually more damaging than continuous treatment (7).

Data extracted from the excitation-emission matrices for thermally treated Piedmont and dark brown hair are provided in Table III. For dark brown hair, we observe a decrease in the residual Trp levels while the I_{Kyn} and I_{509} bands are statistically similar when comparing the thermally exposed region of the tress with the unexposed portion. The calculated peak ratios reveal the same information. In Piedmont hair, thermal exposure results in Trp loss, degradation of kynurenines, and an increase in the intensity at I_{509}. As expected, the ratio, I_{Trp}/I_{Kyn}, decreases in thermally exposed hair as compared to the unexposed region of the tress. In contrast, there is an increase in I_{509}/I_{Kyn} in thermally exposed Piedmont hair. We suspect that the formation of yellow coloration in thermally exposed hair is responsible for the observed increase in this ratio.

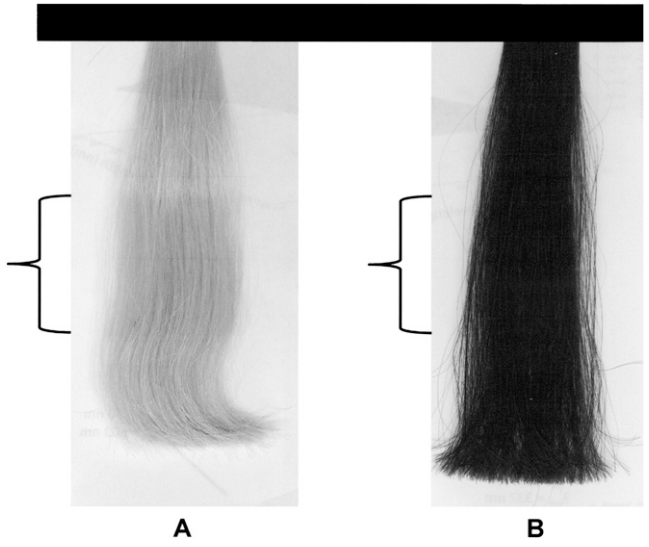

Figure 4. Photographs of thermally exposed (A) Piedmont and (B) dark brown hair.

Table III
Peak Intensity Values and Pertinent Peak Ratios for Dark Brown and Piedmont Hair Exposed to Thermal Treatment

	I_{Trp}	I_{Kyn}	I_{509}	I_{Trp}/I_{Kyn}	I_{509}/I_{Kyn}
Dark brown	24,550 ± 2,616	103,050 ± 5,586	33,250 ± 7,283	0.239 ± 0.038	0.321 ± 0.053
Dark brown-thermal	15,100 ± 849	108,000 ± 4,243	29,650 ± 4,172	0.140 ± 0.002	0.276 ± 0.049
Piedmont	44,600 ± 5,657	2,030,000 ± 98,995	1,275,000 ± 63,640	0.022 ± 0.001	0.621 ± 0.062
Piedmont-thermal	18,900 ± 1,838	1,800,000 ± 127,279	1,540,000 ± 28,284	0.010 ± 0.000	0.858 ± 0.076

Data are provided for the unexposed and exposed regions of the hair tress.

PHOTODAMAGE OF HAIR

As part of the integument, the outermost organ of the body, hair is constantly exposed to solar radiation. Normally, UVB radiation (290–320 nm) is of great concern due to it deleterious effects in the skin resulting in DNA damage, possibly leading to carcinomas or melanoma. Historically, UVA radiation (320-400 nm) was thought to be less significant since its light waves are much lower in energy than UVB radiation. In the last decade, more emphasis has been placed on the recognition that UVA is also damaging (greater quantities reach the Earth's surface) and causes a number of free radical reactions that lead to lipid peroxidation, protein degradation, and even, DNA damage in skin. In hair, lipid peroxidation, can result in altered surface properties and structural organization while protein degradation affects the overal morphological integrity of the fiber.

As already noted, measurement of Trp by spectrofluorescence was employed to monitor the health state of hair exposed to solar radiation (10). In the current study, we utilize similar methodology; however, we generate excitation-emission matrices in order to probe the behavior of all fluorophores present in hair. Figure 5 contains a photograph of a hair tress exposed to 96 hours of UV radiation. As noted in the figure, only the middle section of the tress was exposed, resulting in photobleaching of the yellow pigment naturally present in Piedmont hair. An excitation-emission matrix for the photo-exposed Piedmont hair is presented in Figure 6. Similar to what we observed in bleached hair, photoexposure reduces the peak intensity for the bands at longer excitation wavelengths. In untreated Piedmont hair, there is convergence of numerous bands starting from the principal kynurenine peak (λ_{ex} = 394 nm, λ_{em} = 465 nm) to the longest excitation wavelength (λ_{ex} = 450 nm, λ_{em} = 509 nm). After photo-exposure, the bands at longer wavelengths decrease relative to the principal kynurenine band. This same effect was observed in bleached hair (see Figure 3) and coincides with a loss of the natural yellow hue of Piedmont hair. A summary of the data from excitation-emission matrices for dark brown and Piedmont hair is provided in Table IV. For all of the monitored fluorophore bands (I_{Trp}, I_{Kyn}, and I_{509}), we see a decrease in intensity when comparing the unexposed to exposed regions. In the case of dark brown hair, I_{Trp}/I_{Kyn} increases in the region of the tress exposed to UV light whereas in Piedmont hair similar readings are obtained for both regions. At first glance, one may speculate that this is a melanin-associated phenomenon—the kynurenines are protected more than Trp by melanin in dark brown hair. In terms of absolute absorbance, we would expect the contrary to be true since melanin absorbance is greater

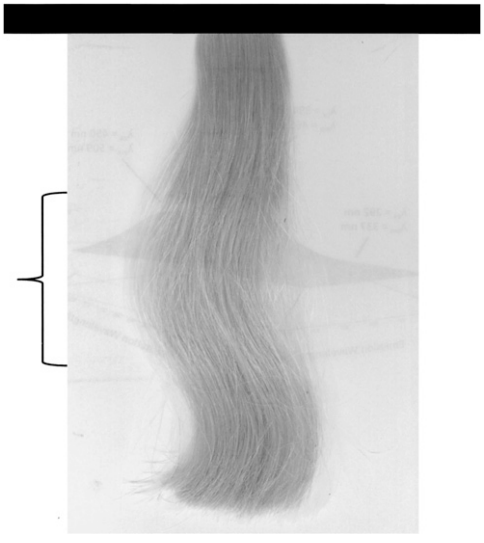

Figure 5. Photograph of Piedmont hair exposed to 96 hours of UV radiation.

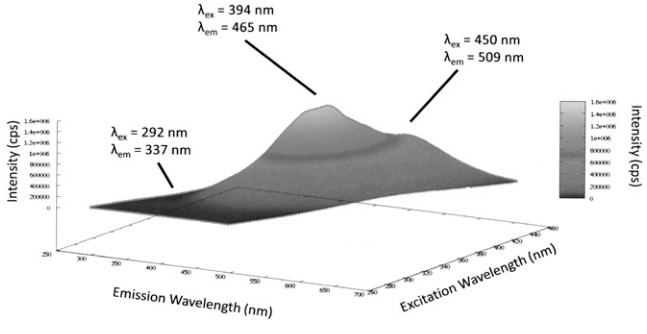

Figure 6. Excitation-emission matrix of Piedmont hair exposed to 96 hours of UV radiation.

at lower wavelenths and monotonically decreases with increasing wavelenth. One must bear in mind that photo-degradation is a kinetic process resulting in the degradation and formation of various fluorophores. In this experiment we only examine the end point. The results clearly indicate the need for further kinetic studies.

In order to better understand the susceptibility of hair to photo-damage in the presence and absence of melanin and other chromophores, we subjected bleached Piedmont and dark brown hair to UV radiation. The data obtained from the excitation-emission matrices is provided in Table V. Similar to what we observe in non-bleached hair, all of the principal fluorescence bands (I_{Trp}, I_{Kyn}, and I_{509}) decrease in intensity after exposure to UV radiation. In the case of non-bleached dark brown hair (Table IV), we found that I_{Trp}/I_{Kyn} decreased when comparing unexposed to exposed regions of the tress. In the case of bleached dark brown hair (Table V), I_{Trp}/I_{Kyn} is statistically equivalent for the exposed and unexposed regions of the tress. We also find that I_{509}/I_{Kyn} increases after exposure for bleached dark brown hair. Both ratios for Piedmont hair are essentially the same (within the standard deviation) when comparing the exposed and unexposed regions of the tress.

Table IV
Peak Intensity Values and Pertinent Peak Ratios for Dark Brown and Piedmont Hair Exposed to UV Radiation

	I_{Trp}	I_{Kyn}	I_{509}	I_{Trp}/I_{Kyn}	I_{509}/I_{Kyn}
Dark brown	31,200 ± 2,121	86,150 ± 495	26,500± 0	0.362 ± 0.022	0.308 ± 0.001
Dark brown-UV	25,850 ± 1,061	55,650 ± 1,202	15,800 ± 566	0.465 ± 0.029	0.284 ± 0.002
Piedmont	37,900 ± 283	1,825,000 ± 91,923	1,300,000 ± 28,284	0.021 ± 0.001	0.713 ± 0.020
Piedmont-UV	29,250 ± 5728	1,440,000 ± 42,426	659,500 ± 33,234	0.020 ± 0.003	0.458 ± 0.010

Data are provided for the unexposed and exposed regions of the hair tress.

Table V
Peak Intensity Values and Pertinent Peak Ratios for Bleached Dark Brown and Piedmont Hair Exposed to UV Radiation

	I_{Trp}	I_{Kyn}	I_{509}	I_{Trp}/I_{Kyn}	I_{509}/I_{Kyn}
Dark brown-bleached	33,150 ± 2,192	237,000 ± 5,657	130,500± 4950	0.140 ± 0.006	0.551 ± 0.034
Dark brown-bleached-UV	24,550 ± 1,344	163,500 ± 707	99,650 ± 3323	0.150 ± 0.008	0.610 ± 0.023
Piedmont-bleached	68,950 ± 4,031	3,075,000 ± 120,208	1,480,000 ± 56,568	0.022 ± 0.000	0.482 ± 0.037
Piedmont-bleached-UV	38,150 ± 1,061	2,170,000 ± 14,142	999,500 ± 99,702	0.018 ± 0.000	0.461 ± 0.049

Data are provided for the unexposed and exposed regions of the hair tress.

It may be more informative to examine the differences between photo-exposed and unexposed regions of the hair tress. These data are provided in Table VI and illustrate the effects of oxidizing melanin in dark brown hair and removing the yellow pigment in Piedmont hair. The difference in I_{Trp} between the unexposed and exposed regions of the tress increase in photo-irradiated hair that is first subjected to bleaching treatment. The same observation was made for I_{Kyn} and I_{509}. In relation to the fluorophores monitored, hair that has undergone bleaching is more susceptible to photo-degradation than the same hair that has not been chemically treated. This is true for both dark brown and Piedmont hair.

CONCLUSIONS

We utilized steady-state spectrofluorescence as an analytical tool to monitor the health of human hair fibers. Hair was subjected to routine consumer stresses such as bleaching, straightening (chemical relaxers), hot thermal irons, and solar radiation. Fluorescence excitation-emission matrices were generated for hair providing a spectroscopic map of all its fluorophores. Two different hair types, Piedmont and dark brown, were utilized to monitor the effects of melanin as a protecting agent and its influence on the fluorescence

Table VI
Peak Intensity Value Differences Between Unexposed and Exposed (unexposed - exposed) Region of the Tress

	I_{Trp}	I_{Kyn}	I_{509}
Dark brown	5,350 ± 1,061	30,500 ± 1,697	10,700 ± 566
Piedmont	8,650 ± 6,010	385,000 ± 134,350	640,500 ± 61,518
Dark Brown-bleached	8,600 ± 849	73,500 ± 4,950	30,850 ± 1,626
Piedmont-bleached	30,800 ± 2,970	905,000 ± 106,066	480,500 ± 43,134

behavior of hair when subjected to physical and chemical stresses. For example, bleaching of both hair types resulted in an increase in the fluorescence signal of Trp, which could be attributed to its greater absorption and subsequent emission in the absence of other chromophores. Hair straightening was found to damage Trp and, ultimately, the kynurenines. Thermal exposure of hair results in a dark yellow discoloration, evident in Piedmont hair, which correlates with an increase in the fluorescence intensity of the kynurenine bands. On the other hand, upon extended photo-irradiation, Piedmont hair was found to lose its natural yellow pigmentation—concurrent with a decrease in the intensity of kynurenine fluorescence.

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