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Tryptophan fluorescence in hair—Examination of contributing factors

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Synopsis

Various types of hair, including white (unpigmented), Piedmont (yellow-colored), blonde, brown, curly black of African origin, straight black of Chinese origin, and chemically processed (bleached) hair, were studied by using fluorescence spectroscopy. Fluorescence measurements were obtained by using a single- or double-grating fluorescence spectrophotometer and a bifurcated fiber optics accessory to measure the spectra directly from the surface of the hair. The results have shown that all types of hair share similar fluorescence characteristics, as recorded by excitation at 290 nm, 320 nm, 350 nm, and 380 nm, with emissions that could be ascribed to chromophores such as tryptophan (Trp), N-formylkynurenine, kynurenine, and 3-hydroxykynurenine. The relative intensities of fluorophore emissions were found to be dependent on factors such as melanin content and the history of UV light or thermal exposure. Trp fluorescence was also found to be dependent upon the state of the hair matrix. Softening of hair keratin by chemical reduction (breakage) of disulfide bonds or by hydration, leads to a 50%–100% increase in Trp emission intensity. Conversely, stiffening of the hair matrix by re-oxidation of reduced hair with hydrogen peroxide, or drying of wet hair, produces a decrease in fluorescence intensity. The results were interpreted by invoking the behavior of certain enzymes, which demonstrate Trp quenching by neighboring disulfide bonds or by hydrogen bonding with alanine residues, or with certain side-chain amino acids.

INTRODUCTION

Spectroscopic techniques have been frequently employed for the characterization of chemical and physical processes related to hair. For example, reactions occurring during photo and oxidative degradation of hair have been analyzed by IR (1), fluorescence (2), Raman (3), and ESCA (4) spectroscopies. IR analysis was employed to identify the oxidation products of sulfur-containing amino acids while Raman spectroscopy may be utilized for simultaneous analysis of tryptophan decomposition, disulfide bond breakage, thiol formation, and the appearance of oxidation products. Fluorescence proved to be very convenient in monitoring the photodecomposition of Trp as a result of exposure to solar radiation. These studies were primarily concerned with Trp emission, which is observed at wavelengths in the range of 335 nm to 350 nm. Since the absorption maximum of Trp is at 280 nm, it partially overlaps the UV-B region of solar radiation, which is related to a number of hair damage reactions such as surface roughening due to photodecomposition of surface lipids or loss of mechanical strength. Thus, decomposition of Trp itself can be employed as an indicator of hair photo-exposure and damage.

However, the potential of Trp fluorescence has not been fully exploited and can be further extended to study the UV-A and visible regions (from 320 nm to 500 nm) of the absorption and emission spectra of hair (Table I). In this range of wavelengths, we analyze products of Trp decomposition, such as kynurenines, rather than Trp itself. The spectroscopic changes in hair occurring in this wavelength range are important for cosmetic applications such as hair UV protection and hair dyeing. Furthermore, they can be influenced by the adsorption or deposition of UV-A photofilters, dyes, pigments, or natural products such as melanin. In addition, Trp fluorescence can be affected by chemical or physical modification of hair. As discussed in this paper, waving and re-oxidation, alkaline relaxing, or simply changing the content of water in hair can have a significant effect on the position and intensity of Trp emission. This is due to the fact that the position and the intensity of the fluorescence of Trp residues are very sensitive to their chemical environment in the protein matrix (5).

In the current investigation, we concentrate on the analysis of Trp-related spectral changes in hair as a result of photoirradiation, exposure to elevated temperatures, and selected chemical and physical treatments. Hair samples with different degrees of pigmentation, such as unpigmented Piedmont hair, Caucasian dark brown hair, and black-colored Asian hair (Chinese origin) were analyzed by the use of fluorescence spectroscopy. The report also discusses the effect of melanin on the fluorescence spectra of hair.

EXPERIMENTAL

MATERIALS AND METHODS

All fiber samples, consisting of Piedmont hair as well as blonde, Caucasian dark brown, and Asian (Chinese origin) hair, were commercially blended and purchased from DeMeo Brothers, New York. African and unpigmented white hair were purchased from International Hair Importers (Glendale, NY). Commercially bleached hair was obtained from DeMeo Brothers (fluorescence spectrum obtained by a single-grating Fluorolog-2 instrument is shown in Figure 2f) and from International Hair Importers (fluorescence spectrum obtained by double-grating Fluorolog-2 instrument is shown in Figure 3). Bleached hair was also prepared in-house by reacting dark brown hair with a 1:1 paste obtained by

Table I
Hair Chromophores

Chromophore	Absorption (nm)	Emission (nm)
Melanin	200–600	Weak
Trp	285	330–350
N-Formylkynurenine	320	420
Kynurenine	350–360	460
3-Hydroxykynurenine	365	495
Tyrosine	275 nm	306, 392
Phenylalanine	260	302
Cystine	<200	465
Artificial hair color	200–600	Weak

mixing a bleaching powder (Clairol BW2 powder bleach) and hydrogen peroxide (Clairol 20 volume cream developer). The hair was precleaned with 3% ammonium lauryl sulfate solution prior to use in experiments. Sepia, synthetic melanin, L-Trp, and L-kynurenine were purchased from Sigma. Lipomelanin and water-soluble melanin were obtained from Mel-Co.

INSTRUMENTATION

Fluorescence measurements were obtained by using a Fluorolog-2 fluorescence spectrophotometer (Model 212, Spex Industries, Edison, NJ) and a bifurcated fiber optics accessory to collect the spectra directly from the surface of hair by delicately pressing the probe against the hair lying on a solid, black-colored support to produce good contact and eliminate stray illumination No special procedures were required to prepare the fibers for fluorescence measurements. Some measurements, as indicated further on in the text of the paper, were carried out using a double-grating Fluorolog-2 fluorescence spectrophotometer. The emission and excitation slits were set at 2-nm bandpasses, with an exception for studies of bleached hair in which a 1-nm bandpass was employed due to higher fluorescence light intensity. The measurements were performed in both emission and excitation modes by irradiating hair in the wavelength range of 260 to 400 nm. This range of wavelengths was monitored in order to probe hair chromophores absorbing the light in various parts of the UV-Vis spectrum.

The UV-Vis absorption spectra of L-Trp (aqueous solution at a concentration of $1.65 \cdot 10^{-4}$ M), L-kynurenine (aqueous solution at a concentration of $2.11 \cdot 10^{-4}$ M), and water-soluble melanin (at a concentration of 0.03 mg/ml) were collected using a Perkin-Elmer Model 559A spectrophotometer. Hair fibers were photoirradiated by artificial radiation in an Atlas weatherometer, which employed a xenon lamp to simulate solar radiation. Thermal treatment of hair was carried out using a Soft Sheen, Optimum Styling Tools, curling iron (Model SOC 125 S) manufactured by Continental Hair Products (Glendale, AZ).

RESULTS AND DISCUSSION

FLUORESCENCE SPECTRA OF VARIOUS TYPES OF HAIR

The absorption and emission characteristics of keratin fibers is dominated by the presence of the pigment melanin and the absorbing residues of the protein structure, such as the amino acid Trp along with its oxidation products, i.e., kynurenines (6–14).

A typical solubilized melanin absorption spectrum is characterized by a monotonic increase in absorbance with decreasing wavelength as shown in Figure 1 (15–17). The absorption maxima of Trp and kynurenine lie at approximately 285 nm and 360 nm, respectively (Figure 1). A scheme presenting the conversion of Trp into its oxidation products, 5-hydroxytryptophan, N-formylkynurenine, kynurenine, and 3-hydroxykynurenine, is shown in Scheme 1 (14).

The fluorescence spectra of unpigmented white hair, white hair with a yellow tinge (Piedmont), blonde hair, highly pigmented dark brown hair, black African hair, and bleached

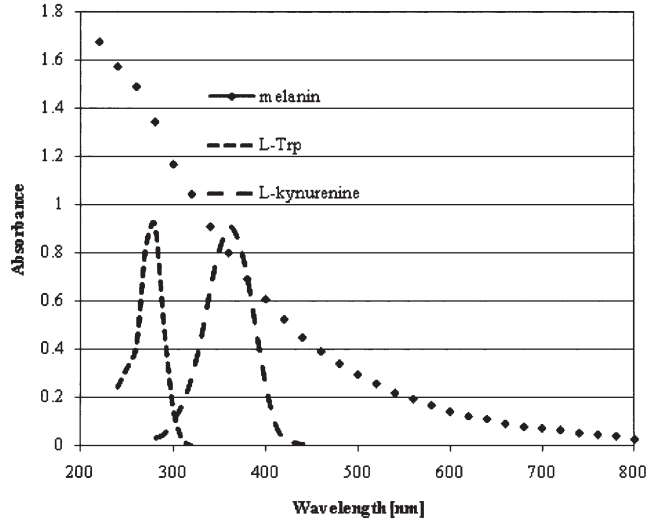
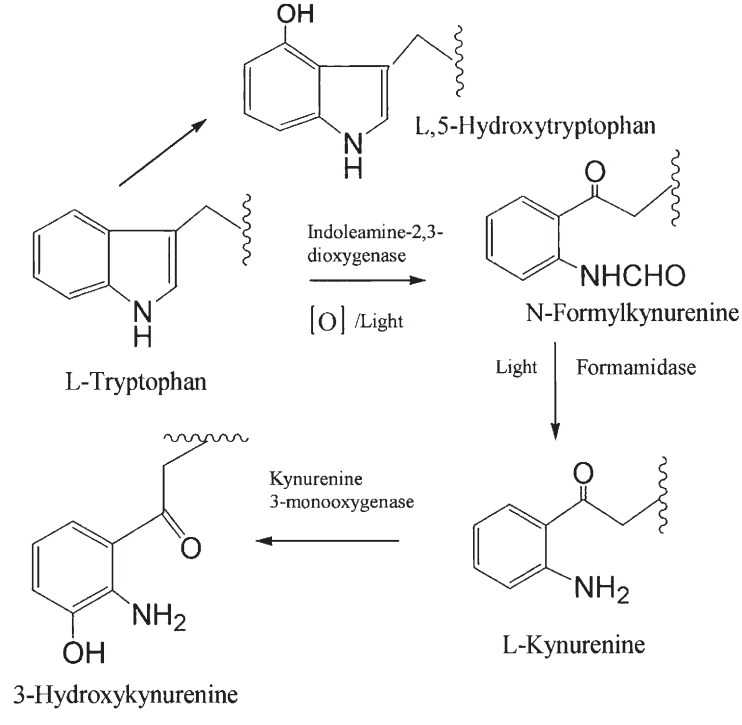


Figure 1. UV-Vis absorption spectra of selected hair chromophores.

hair are presented in Figure 2(a-f). The spectra were obtained using excitation wavelengths of 290, 320, 350, and 380 nm in order to probe various chromophores present in the fiber structure. Three distinct peaks are evident in the spectra of all types of hair. Excitation at 290 nm, i.e., at the maximum of Trp absorption, produces a strong band at



Scheme 1. Metabolic and photo-oxidation products of tryptophan (14).

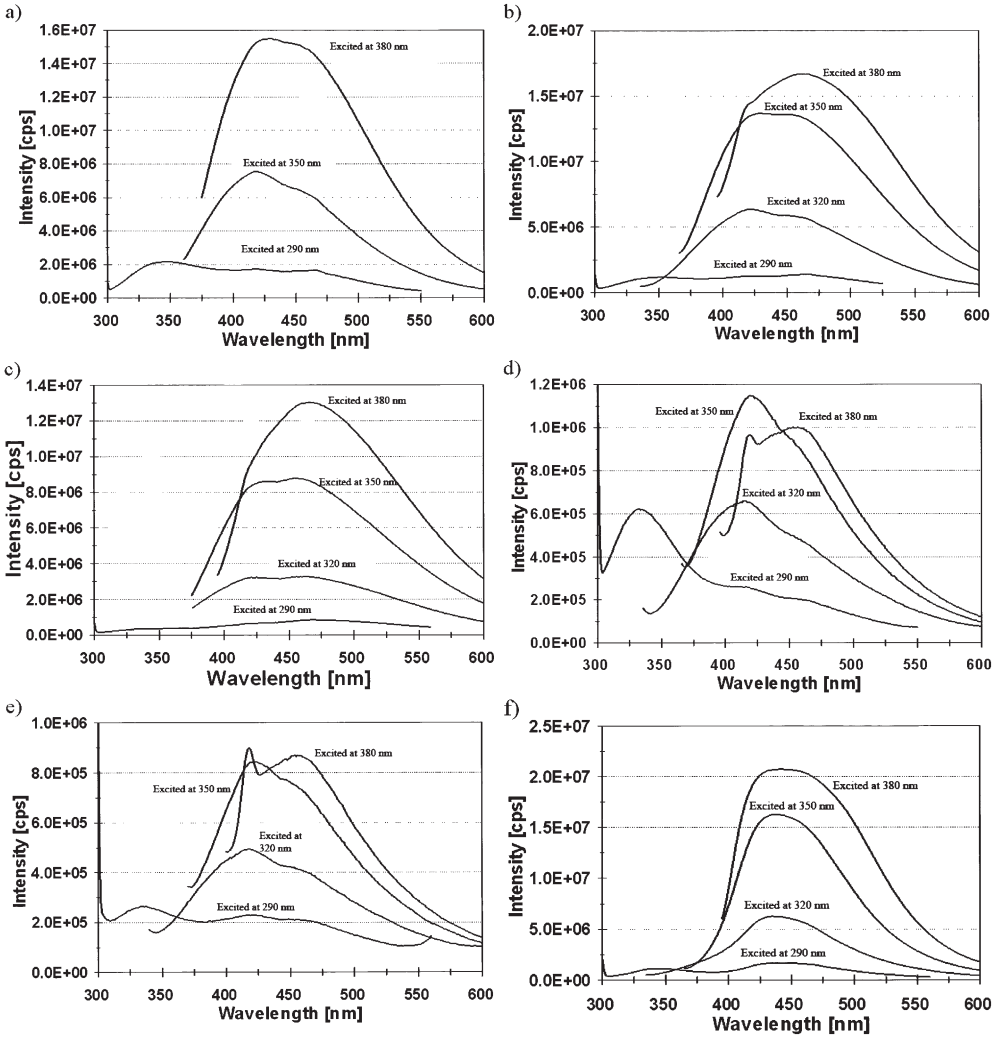


Figure 2. Fluorescence spectra of (a) white hair, (b) Piedmont hair, (c) blonde hair, (d) brown hair, (e) African hair, and (f) bleached hair.

335–350 nm, which corresponds to the fluorescence of Trp (2). The maximum of this peak shifts towards shorter wavelength with a decrease of melanin pigmentation. The emissions at 420 nm, 465 nm, and 495 nm are due to N-formylkynurenine, kynurenine, and 3-hydroxykynurenine, respectively (7–14). The peaks from 3-hydroxykynurenine and L-kynurenine were previously identified by comparing the spectrum of hair with the spectra of pure solid L-kynurenine (emission maximum at 465 nm) and 3-hydroxykynurenine (emission maximum at 495) (14,15). The emission peak at 395 nm, evident in spectra obtained by a double-grating instrument, was not identified. It should be pointed out that all types of the investigated hair, including Chinese hair not shown in Figure 2, share similar emission characteristics with the most prominent emission peaks corresponding to Trp and kynurenines.

The emission spectra of the solid samples of three different melanins, including sepia, synthetic melanin, and lipomelanin, are very similar and show a relatively weak fluorescence with small maxima at 417, 468, and 609 nm for an excitation wavelength of 380 nm. The strongest band has a maximum at 417 nm and is characterized by intensity in the range of $1\text{-}2\cdot10^5$ cps depending on the melanin sample. The analysis of the spectra obtained at excitation wavelengths of 290, 320, and 350 nm did not show any additional features of the fluorescence characteristics of melanin (15–17). Considering the relatively low intensity of the fluorescence of intact melanin, it can be probably assumed that its direct contribution to the emission characteristics of hair is minor. On the other hand, it may be expected that the high incident light absorption by melanin pigment and its high concentration in dark-colored hair can significantly reduce the emission intensity of protein chromophores such as Trp and the kynurenines.

The above hypothesis evolved from experimental data showing that the intensity of the fluorescence emission from unpigmented hair is much higher than that from pigmented hair (14). For Trp emission due to excitation at 290 nm, the maximum fluorescence intensity is $1.2\cdot10^6$, $6.1\cdot10^5$, and $6.6\cdot10^5$ cps for unpigmented, dark brown, and black-colored hair, respectively. For spectra obtained at higher excitation wavelengths, the difference in emission intensity between unpigmented and pigmented samples is even larger. For an excitation wavelength of 350 nm, the emission intensities at 420 nm (ascribed to N-formylkynurenine) were $1.4\cdot10^7$, $9.0\cdot10^5$, and $1.1\cdot10^6$ cps for Piedmont, dark brown, and black-colored Chinese hair, respectively.

The spectral features of bleached hair excited at 290 nm include a band at 345 nm, with a maximum intensity of $1.1\cdot10^6$ cps corresponding to the emission of Trp, and broad peak with a maximum at 435 nm and an intensity of $1.7\cdot10^6$ cps (Figure 2f). The shape of this peak suggests that it consists of several bands including those corresponding to the kynurenines (420 nm and 465 nm) and possibly due to melanin decomposition products as well. It was reported that the melanin intermediates formed as a result of its bleaching (and solubilization) by the reaction with hydrogen peroxide were found to be highly fluorescent (15–17). Since the melanin decomposition products are present in bleached hair, as evident by the thermally induced re-melanization reaction, they can probably contribute to the observed emission band at 435 nm. Similar to the spectrum of Piedmont hair, the intensity of this peak increases to $6.27\cdot10^6$, $1.6\cdot10^7$, and $2.1\cdot10^7$ cps for excitation wavelengths of 320, 350, and 380 nm, respectively.

Further analysis of bleached hair was carried out by using a double-grating fluorescence instrument, which provides higher resolution spectra than a traditional, single-grating spectrophotometer. A comparison of the fluorescence spectra of bleached, Piedmont, and dark brown hair, obtained by excitation at 290 and 320 nm, is shown in Figures 3a and 3b. It should be noted that these results were obtained for so-called blended hair, i.e., hair collected from many individuals. The spectra show Trp and kynurenine bands with a high ratio of kynurenine/Trp ($I_{440}/I_{356}=1.78$) for yellow-colored Piedmont hair. This value is as high as the highest ratio previously recorded for unpigmented hair collected from a single panelist. As shown previously for a population of eleven panelists, this ratio can vary from 0.36 to 1.78 and is probably related to environmental and metabolic factors. For the blended dark brown hair employed in the present work, the ratio, I_{440}/I_{356}, was 0.6—approximately in the middle of the range of values previously recorded for pigmented hair derived from a single panelist (14). In the case of bleached hair, the ratio I_{440}/I_{356} was 0.78. It should also be pointed out that Piedmont hair emits higher intensity

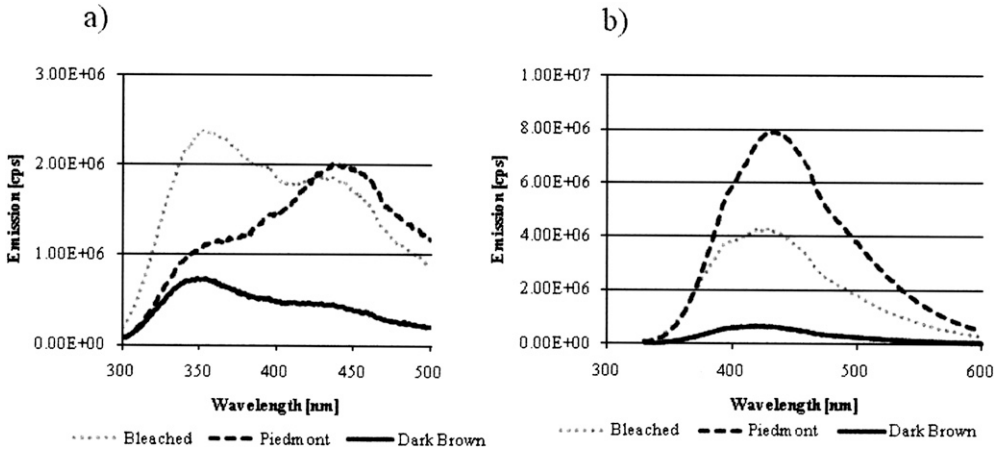


Figure 3. Comparison of the fluorescence spectra of bleached, Piedmont, and dark brown hair excited at (a) 290 nm and (b) 320 nm.

fluorescence corresponding to kynurenine (460 nm) and 3-hydroxykynurenine (495 nm), relative to N-formylkynurenine (420 nm), as compared to bleached hair. This is based on the analysis of the spectra obtained at an excitation wavelength of 320 nm (Figure 3b). The corresponding ratios of I_{460}/I_{420} and I_{495}/I_{420} vary from 0.86 and 0.52 for Piedmont hair to 0.79 and 0.46 for bleached hair. These results suggest that photodegradative or metabolic processes leading to the formation of kynurenine derivatives are more advanced in Piedmont hair.

We have also followed the time dependence of the spectral changes during bleaching. The results are presented in Figures 4a and 4b and they illustrate (a) a general increase in the fluorescence intensity as a function bleaching time and (b) a relative increase in fluorescence intensity corresponding to kynurenines vs. Trp (Figure 4a).

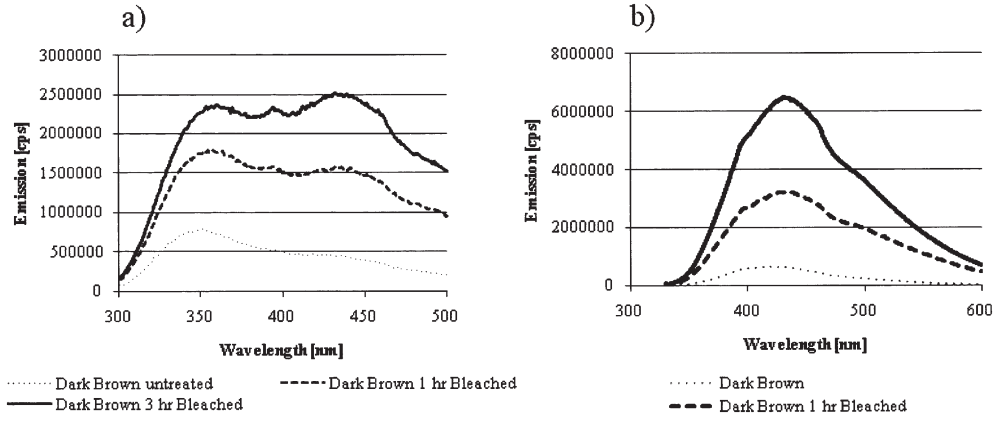


Figure 4. Effect of bleaching time on the fluorescence spectra of hair obtained at the excitation wavelength of (a) 290 nm and (b) 320 nm.

Figure 5 presents emission spectra of intact and photo-exposed Piedmont hair, which were obtained at an excitation wavelength of 290 nm. A decrease in the intensity of the peaks at 350 nm (43%), 420 nm (27%), and 465 nm (23%) as a result of 24-hour irradiation in a weatherometer is evident, suggesting photodecomposition of Trp and the kynurenines. It should be noted that a decrease in the Trp emission is significantly larger than the corresponding reductions in the intensity of the peaks at 420 and 465 nm, which may be related to the phenomenon that kynurenines are first formed as a result of Trp photo-oxidation reactions before undergoing subsequent photodecomposition. Further examination of fluorescence spectra obtained at an excitation wavelength of 350 nm confirms the decomposition of kynurenines by showing reductions of peak intensities at 420 nm (12%) and 465 nm (17%). Similar trends were observed in the spectra of Piedmont hair subjected to thermal treatment with hot irons at 160°C (15,19). A decrease in the intensity of peaks corresponding to both Trp and kynurenine emissions was observed. In the case of thermally treated hair (160°C for 30 minutes), an increase in the ratio of I_{465}/I_{420} (from 1.12 for intact hair to 1.37 for thermally treated hair, as calculated from the spectra obtained at an excitation wavelength of 290 nm) was noted, which may reflect predominant formation of kynurenine, which could in turn be responsible for the yellow coloration of thermally treated Piedmont hair.

The results of the fluorescence analysis of photodegradation of various types of hair as a result of irradiation for 72 hours are collected in Table II. White and Piedmont hair showed the largest loss of Trp fluorescence (in the range of 56.7% to 64.8%), while the corresponding decreases for highly pigmented dark brown and Asian hair were significantly smaller (47.7% to 42.9%). For kynurenines, the fluorescence decreases were smaller especially for highly pigmented Chinese and dark brown hair. It should also be mentioned that for dark brown and black Chinese hair the analysis of the spectra resulting from excitation at 350 nm actually shows an increase in the intensity of the peaks ascribed to kynurenines (for dark brown hair, an increase of 11% and 14% at 420 and 465 nm, respectively). This result may suggest that the melanin, present in dark-colored hair, offers selective photoprotection to kynurenines by decreasing the extent of their photodecomposition. It should also be added that the dark brown hair employed in this work was

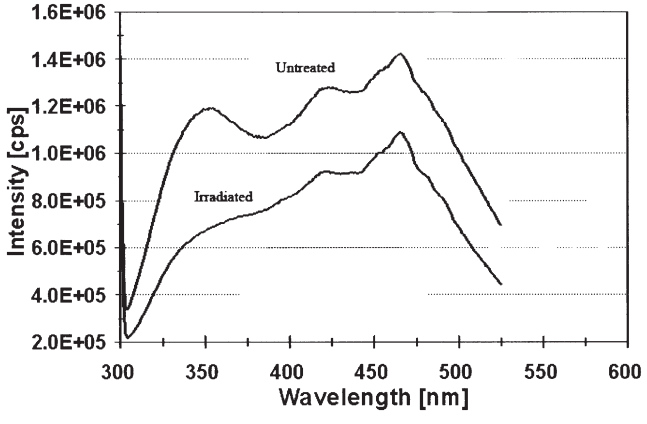


Figure 5. Comparison of the fluorescence spectra of Piedmont hair, untreated and irradiated for 24 hours.

highly pigmented and visually appeared to be as dark as black Chinese hair. An alternative explanation of this effect could be that the photobleaching of melanin, which also takes place during exposure of hair to light, results in higher absorption and thus higher fluorescence emission by the keratin chromophores.

The behavior of light brown hair was similar. In contrast, bleached hair lost very little Trp, while its fluorophores at longer wavelengths (420 and 465 nm) experienced more damage than was the case with other hair types. The behavior of bleached hair is complex since it undergoes very significant transformations in the bleaching process, leading to not only melanin removal, but also oxidation of the keratin protein. A detailed explanation of the photodegradation mechanism of bleached hair is beyond the scope of this paper and will be addressed in future work.

Finally, we analyzed the effect of externally applied melanin (as a leave-in or rinse-off solution) on the fluorescence spectra of hair. The results, presented in Figure 6, show that a leave-in deposition of lipomelanin on white hair results in a reduction of hair fluorescence both in the Trp (330–350 nm) and kynurenine (400–500 nm) regions of the emission spectra. Furthermore, the fluorescence spectrum of pigmented, light brown hair was found to be very similar to that obtained from white hair externally coated with 2% lipomelanin. Such a result suggests that a simple light-absorption mechanism, rather than

Table II
Results of Fluorescence Analysis of Intact and Photoirradiated Hair with Various Degrees of Pigmentation

Hair	I (350) (cps) Exc. 290 nm	% Trp loss	I (420) (cps) Exc. 350 nm	I (465) (cps) Exc. 350 nm	% 420 nm loss	% 465 nm loss
White	$2.4{\cdot}10^6$	56.7	$1.6{\cdot}10^7$	$1.5{\cdot}10^7$	21.5	26.9
Piedmont	$1.6{\cdot}10^6$	64.8	$1.4{\cdot}10^7$	$1.4{\cdot}10^7$	25.5	30.3
Bleached	$1.1{\cdot}10^6$	30.2	$1.9{\cdot}10^7$ (431 nm)	$1.9{\cdot}10^7$ (460 nm)	32.6	32.2
Light brown	$0.8{\cdot}10^6$	38.8	$8.5{\cdot}10^6$	$8.7{\cdot}10^6$	9.1	5.7
Dark brown	$0.7{\cdot}10^6$	47.7	$1.1{\cdot}10^6$	$0.8{\cdot}10^6$	3.0	4.0
Asian	$0.6{\cdot}10^6$	42.9	$1.7{\cdot}10^6$	$1.3{\cdot}10^6$	13.3	17.9

Irradiation was carried out in a weatherometer for 72 hours.

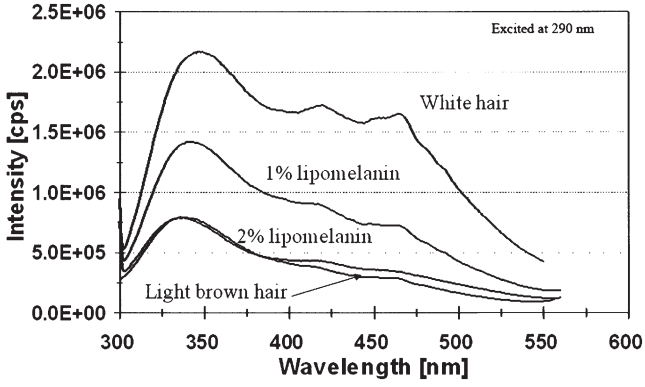


Figure 6. Effect of lipomelanin coating on the fluorescence spectra of white hair.

energy transfer between hair and melanin chromophores (which would require intimate contact between melanin and protein chromophores), is responsible for the observed quenching of hair fluorescence. It is also important to point out a blue shift in the maximum emission of Trp from 350 nm for white hair, to 342 nm and 336 nm for hair coated with 1% and 2% lipomelanin, respectively. As shown in the previous section, a similar shift is observed in the fluorescence spectra of hair with increasing amounts of melanin pigmentation, i.e., upon comparison of the spectra for unpigmented white or Piedmont hair with those corresponding to brown- or black-colored hair.

We also carried out experiments to test the photoprotective properties of externally applied melanin to hair. The results of fluorescence analysis of white hair irradiated in a weatherometer are collected in Table III. The loss and protection parameters were calculated based on the fluorescence peaks corresponding to Trp and kynurenine. The effect of melanin application followed by rinsing was found to be minimal in the case of Trp and slightly higher for UV-A chromophores. For leave-in treatments the extent of photoprotection was significant and ranged from 24% for Trp to as high as 55.8% for kynurenine in the case of 1% melanin application. The calculated loss and protection values for kynurenines were actually higher for 1% melanin than for 2% melanin treatment. This finding suggests saturation of a protective effect for relatively photoresistant kynurenine chromophores.

EFFECT OF CHEMICAL TREATMENTS

Chemical modification of hair by reducing agents was found to produce a dramatic increase in the Trp fluorescence of hair. We have carried out experiments employing sodium and ammonium thioglycolates, which are most frequently used in hair waving products. Both reducing agents react with disulfide bonds at pH 8–9.5, leading to a symmetric scission and formation of two thiol groups. The thiols can be re-oxidized by reaction with hydrogen peroxide, thereby reconstituting the cleaved disulfide bonds.

Table IV compares the results of the fluorescence analysis for untreated hair and for hair treated with sodium thioglycolate (NaTGA), ammonium thioglycolate (NH_4TGA), sodium hydroxide, and a commercial perm based on ammonium thioglycolate. The data demonstrate a large increase in the fluorescence intensity of Trp—about 40–50% after hair reduction. The application of a commercial perm, which represents a complete formulation (the concentration of the reducing agent is unknown; the formula also contains surfactants and emulsifying agents), led to an increase in Trp fluorescence intensity of

Table III
Results of Fluorescence Analysis of Trp and Kynurenine Photodegradation of Untreated and Lipomelanin-Treated White Hair

Treatment	% Trp loss/ protection	% 420 nm loss/ protection	% 465 nm loss/ protection
Untreated	56.7/0	21.5/0	26.9/0
1% Melanin rinse-off	56.0/1.2	20.6/4.2	25.7/4.5
1% Melanin leave-in	43.1/24	12.0/44.7	11.9/55.8
2% Melanin leave-in	35.3/37.7	16.4/23.7	14.6/45.7

Table IV
Effect of Chemical Treatments on Trp Fluorescence of Dark Brown Hair

Hair treatment	Intensity of Trp fluorescence at 336 nm × 10^5 (cps)	
	Untreated	Treated
6% NaTGA, pH 9.0 (15 min)	5.1 ± 0.2	7.8 ± 0.3
6% NaTGA (15 min)/2% H_2O_2 (5 min)	5.4 ± 0.2	6.4 ± 0.2
6% NH_4TGA, pH 9.0 (15 min)	5.1 ± 0.2	8.4 ± 0.3
6% NH_4TGA (15 min)/2% H_2O_2 (5 min)	5.3 ± 0.2	6.4 ± 0.3
3% NaOH (15 min)	5.6 ± 0.3	2.4 ± 0.2
Commercial perm (NH_4TGA) (15 min)	5.2 ± 0.2	10.1 ± 0.5
Perm and neutralizer (15 min and 5 min)	5.2 ± 0.2	6.9 ± 0.2

The spectral analysis was carried out on dry hair at ambient conditions (40%–50% RH).

nearly 100%. The results presented in Table IV also include the analysis of hair that was first reduced and subsequently re-oxidized with hydrogen peroxide. The data suggest a decrease in the fluorescence intensity of Trp after treatment with hydrogen peroxide, as compared to reduced hair, to levels about 20% higher than the initial values obtained for untreated hair. Based on these data, one can conclude that the reduction–re-oxidation cycle does not reconstitute the original structure of untreated hair, at least as probed by Trp fluorescence.

In contrast to thioglycolates, which act as active reducing agents at pH 9, the use of 3% sodium hydroxide leads to a decrease in the intensity of Trp fluorescence from $5.6 \pm 0.3 \cdot 10^5$ cps to $2.4 \pm 0.2 \cdot 10^5$ cps. The treatment of hair with high concentration alkalis such as 3% NaOH is employed in the process of relaxing curly, African hair. The chemistry of this process includes the reaction of HO^- with disulfides and subsequent formation of thiolate groups. Further transformations result in the formation of lanthionine groups and lysinoalanine residues. Disulfide bond cleavage is significant and was reported to be as high as 72% (20). In relaxed hair, disulfides are replaced with lanthionine groups, which maintain the stiffness and rigidity of the keratin matrix.

A possible interpretation of these observations is suggested by reviewing the literature of protein model systems. One of them is a protein referred to as *fusarium solani pisi cutinase*, which is an enzyme with a single L-Trp, which is located close to a disulfide bridge (21). It is also involved in a hydrogen bond with an alanine residue (22). According to Martinho *et al.* (23), there are both static and dynamic quenching mechanisms of the fluorescence of this Trp residue. Disruption of the Ala-Trp hydrogen bond, which occurs during melting of the protein, releases Trp from the viscinity of a cystine residue and results in a fourfold increase in Trp fluorescence intensity. In addition, UV light, with wavelengths corresponding to the maximum of Trp absorption, breaks down the disulfide bridge, resulting in a ten-fold increase in Trp fluorescence quantum yield. The cleavage of the disulfide bond increases the internal backbone mobility of the neighboring residues and releases the Trp residue from the viscinity of the cystine residue (24). Reduction of disulfides in the process of hair waving with thioglycolates has a similar effect. It also has a plasticizing effect on the amorphous matrix in cortical cells and increases the mobility of protein chains. Re-oxidation of cysteine residues into disulfides, as a result of treatment with hydrogen peroxide, reconstitutes the network of disulfide bridges and, consequently, brings about the reduction of Trp emission intensity. It should also be added that other

amino acid side-chain groups, such as those corresponding to aspartate, glutamate, lysine, aspargine, and glutamine, can also quench Trp fluorescence if located within 10 Å from the residue. This may also lead to decreases in Trp fluorescence in hair containing a network of unreduced disulfide bonds.

EFFECT OF WATER CONTENT

Figure 7 shows the fluorescence spectra of brown hair with different levels of hydration. Completely dry hair, immediately after hair dryer application and cooled down to room temperature, displayed the lowest intensity of Trp emission at $5.9 \cdot 10^5$ cps at 336 nm. For completely wet hair the maximum emission was red-shifted to 343 nm and the intensity increased to $9.7 \cdot 10^5$ cps. For hair equilibrated at 50% RH, the observed emission position and its intensity were intermediate between dry and wet hair. It should also be pointed out that while the position and emission intensity of Trp fluorescence is significantly affected by the content of water in hair, the peaks corresponding to kynurenines are not sensitive to such changes in the hydration of hair. This is evident from the fluorescence spectra shown in Figure 5 (excited at 290 nm) and was also confirmed by spectra obtained by excitation at 320 nm, which showed no significant change with a variation in the content of water. In addition, we also examined spectra of other types of hair, such as Piedmont and bleached, obtained at various levels of hair hydration. Qualitatively, the observed spectral changes were similar to those discussed above for brown hair.

The effect of water on Trp emission in hair can be explained by hydrogen bond breaking during hydration involving Trp residues, similar to the effect of protein melting that leads to denaturation of cutinase by temperature increase (23). In this case the disulfide bridges remain intact; however, elimination of hydrogen bonds softens the keratin structure and/or helps to release Trp from the vicinity of cystine residues and induces the disruption of Trp-disulfide bridge complexes. Such structural transformations result in increased fluorescence intensity in hair with high water content.

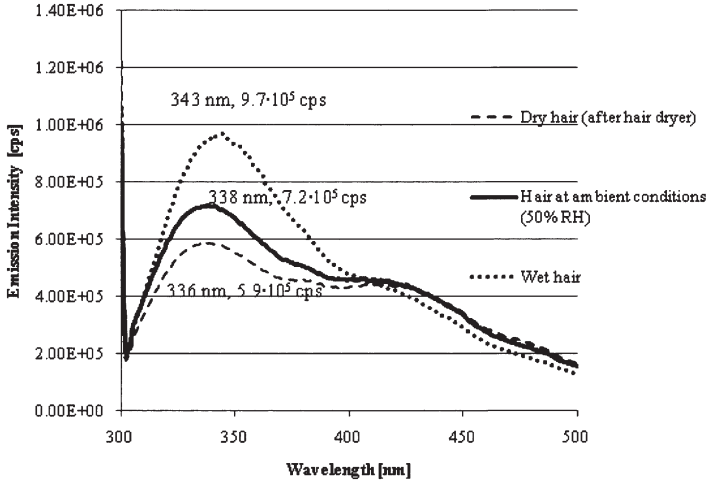


Figure 7. Effect of water content on the fluorescence spectra of brown hair.

CONCLUSIONS

The following conclusions can be drawn based on the findings presented above:

(1) The fluorescence data obtained at several excitation wavelengths and for different types of hair, including white, Piedmont, bleached, blonde, brown, curly black, and Chinese, revealed major evidence for the presence of three major chromophores including Trp and its three photo-oxidation products: N-formylkynurenine, kynurenine, and 3-hydroxykynurenine.

(2) While melanin itself does not seem to contribute significantly to hair fluorescence, the presence of melanin in pigmented hair was shown to reduce the fluorescence intensity of other keratin chromophores.

(3) Photo and thermal exposure of hair was shown to decompose protein chromophores and reduce fluorescence intensity of Trp and, to a lesser extent, kynurenines. The data also point to a photoprotective influence of melanin on kynurenines. Externally applied melanin was also shown to exert a photoprotective effect for other hair chromophores.

(4) Chemical reduction of hair by thioglycolates was shown to increase Trp fluorescence. It was proposed that the effect is caused by softening of the keratin matrix accompanied by an increase in the mobility of protein chains and a consequent decrease in Trp quenching by disulfides. Re-oxidation of thiol groups into disulfides by hydrogen peroxide was demonstrated to decrease Trp fluorescence to prereduction levels. A similar effect is produced by hair hydration, with the magnitude of Trp fluorescence reversibly increasing with water content.

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