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Hair photodamage—Measurement and prevention

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Synopsis

A spectroscopic technique, based on the intrinsic fluorescence of hair keratin, has been used to monitor the concentration of tryptophan in hair. Experiments performed by using either artificial light or natural sunlight have shown that tryptophan is photolabile, and undergoes decomposition on exposure to UV light in the 295- to 315-nm range. Hair weathering was found to produce significant loss of tryptophan, together with other chemical changes in the keratin structure such as the oxidation of disulfide bonds to cysteic acid. Thus it is shown that tryptophan in hair can serve as a sensitive marker of hair photodamage. It is also demonstrated that hair care formulations containing sunscreens can reduce the extent of photodamage. Furthermore, the tryptophan decomposition rate was found to be substantially slower in mineral oil-treated hair, compared to water-treated control. These data suggest that hair care formulations containing non-chromophoric material may also reduce photodamage.

INTRODUCTION

Hair fibers are made up of fibrous proteins belonging to the keratin family. Minor contribution to the total hair mass derives from melanin pigment and lipids. Morphologically, hair structure has two distinct components. The shingle-like cuticles form the hair exterior and enclose the corticular mass. The latter consists of tightly packed elongated cortical cells oriented parallel to the fiber axis. These cells contain α-helical microfibrils in a supramolecular motif, embedded in a cystine-rich amorphous protein matrix. These different protein components comprising the hair fiber have distinctly different amino acid composition (1–3).

Recent reports on the harmful effects of sunlight on human skin have raised the awareness of the deleterious effects of sunlight on biological tissues in general. Hair, though not viable, can also undergo photodegradation (4), which can contribute significantly to the overall hair damage (5). The lack of viability of hair, however, precludes straightforward measurement of damage based on tissue response analogous to skin erythema. Thus, there is an obvious need for a sensitive intrinsic marker of hair photodamage.

Hair weathering results in discoloration due to melanin bleaching, as well as damage to hair keratin (5). While the pigment decomposition is apparent, the damage to the fiber itself is not easily discernible. The most dramatic effect is found to be the oxidation of

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cystine disulfides to cysteic acid. Although long-term exposure of the fibers to UV light is known to affect their mechanical properties (4,6), even shorter irradiation may produce cosmetically undesirable effects such as a loss of luster or an increase of hair resistance to combing (7). There could also be a more subtle damage at the molecular level, which can initiate a cascade of reactions that may eventually produce undesirable effects.

The essential first event in hair photodamage, as in all photoprocesses, is light absorption by the fiber. Only wavelengths above 290 nm will be consequential in natural photodamage, since shorter wavelength UV light will be effectively filtered out by the stratosphere (8). The most significant chromophores in proteins that absorb in the UVB region are the amino acids, tyrosine (Tyr, $\lambda_{max} = 275$ nm, $\epsilon_{290} = 100\ M^{-1}\ cm^{-1}$), tryptophan (Trp, $\lambda_{max} = 280$ nm, $\epsilon_{290} = 4500\ M^{-1}\ cm^{-1}$), and the disulfide bonds ($\lambda_{max} < 200$ nm, $\epsilon_{290} = 40\ M^{-1}\ cm^{-1}$) (9–11). Phenylalanine, another aromatic amino acid, absorbs only in the UVC region and, therefore, is not likely to participate in the photodamage reactions of proteins. The longer wavelength UVA and visible light are not likely to cause damage directly since they are not absorbed by proteins. Thus, of all the chromophores in keratin, only tryptophan (Trp) absorbs significant UVB (290–320 nm) light and is, therefore, likely to be most photolabile. This has, indeed, been clearly demonstrated in the case of bovine eye lens crystallines (12,13).

It is surprising that Trp has received little attention in hair research, and except for a few reports (14–17), Trp is often missing in the published compilations of the amino acid composition of human hair keratin. The estimates for the Trp content of hair range from 0.2% to 1% (3,14,15,17). Such a significant source-to-source variation may have arisen, at least in part, because of the photolability of this amino acid, which is discussed in this report. On the other hand, a significant body of literature exists on Trp photodamage in weathered wool and on its role in the photo-yellowing of wool (18–20). Fluorescence measurements have also been reported on wool cloth (21,22). Furthermore, examples of the deleterious effects of Trp photochemistry, including sensitization of non-chromophoric amino acids, abound in the protein literature. Thus, Trp seems to play a significant role in the photochemistry of keratins and of proteins in general.

The objective of this work was to study the process of photodecomposition of Trp in human hair by using fluorescence spectroscopy. The underlying motivation was the need to develop a sensitive intrinsic marker of hair photodamage that could then be used to assess the efficacy of hair care formulations designed to provide photoprotection. We show here that Trp, indeed, provides a very sensitive indicator of hair photodamage. Using this assay we show that conventional UVB sunscreens in hair care formulations can provide photoprotection. Furthermore, our data suggest that non-chromophoric compounds, such as mineral oil, also reduce hair photodamage.

EXPERIMENTAL

Experiments were routinely performed on Piedmont hair (Commercial white, DeMeo Brothers, New York) unless otherwise stated. Naturally weathered hair was obtained from a 7-yr-old Caucasian child and was compared to intact hair from the same source. All amino acids were from ICN Pharmaceuticals.

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Fluorescence measurements were performed on an MPF-66 fluorescence spectrophotometer (Perkin Elmer Corp.) using the solid-sample accessory. The spectrophotometer is controlled with a Perkin Elmer 7000 computer running the PECLS software. Besides data acquisition, this software also allows limited mathematical data manipulation. Emission measurements were made with the excitation and the emission slits routinely set at nominal 1- and 5-nm band passes, respectively, to minimize photodamage during the measurements. The sample excitation wavelength was dictated by the experimental design. Typically, for measuring Trp emission, the excitation wavelength was set at 295 nm to avoid contribution from other aromatic residues. All spectra were measured in the ratio mode to correct for lamp intensity fluctuations. The spectra, however, were not corrected for the instrumental response. For strongly fluorescent samples, the emission was reduced using screen attenuators in the light path. In those experiments in which the fluorimeter was also used for initiating photodamage, the excitation slit was opened wide (10-nm band-pass) for the required time. The excitation slit was then cut down to 1 nm prior to measurement of the fluorescence emission.

Typically, hair fibers were moistened with distilled water and finely chopped with a razor blade prior to mounting in the cell. Wet hair is easier to cut, does not build up a static charge during cutting, and makes good optical contact in the cell, which reduces unwanted light scattering during the fluorescence measurements. We have found that a typical run requires ~50 mg of hair. A further increase in the amount of hair did not affect the emission intensity.

Percent Trp damage was calculated as $[(I_0 - I_t)/I_0] * 100$, where I_t and I_0 represent, respectively, the Trp emission intensities at any time t and at zero time (no damage). The emission intensities have been assumed to be directly proportional to Trp concentration.

To evaluate the role of water in hair Trp photodestruction, the hair samples were dried in a vacuum desiccator for four days prior to measurements. Also, the cell chamber in the fluorimeter contained a desiccant. As noted earlier, the spectral quality is quite poor when dry fibers are measured. This is primarily due to the scattering artifacts resulting from poor optical contact between individual fibers and between the quartz front plate and the sample.

FTIR spectra were measured in an IR-PLAN™ microscope (Spectra Tech. Inc.) connected to an FTIR spectrometer (Model 1760-X, Perkin Elmer Corp.). Hair samples, ~5 mm long, were prepared by flattening between two steel plates at 18 KPsi. The sample was then placed between 2-mm NaCl windows of a μ-plan compression cell (Spectra Tech. Inc.). A small crystal of KBr was included with the sample to serve as a reference against which the sample was ratioed. All spectral measurements were made at ambient temperature and humidity, unless otherwise stated.

RESULTS AND DISCUSSION

PHOTODECOMPOSITION

Initial data, suggesting that hair weathering results in damage to tryptophan, were obtained by comparing the fluorescence spectra of the sun-bleached tip ends (blond) and the unexposed root ends (brown) of hair fibers from a 7-year-old Caucasian boy. The

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brown hair of this child, as of many dark-haired people, becomes streaked with blond on the surface in summer. We found that excitation of the pigmented (brown) hair at 295 nm resulted in a strong emission at ~340 nm. This band, however, was absent in the blond hair spectrum, as shown in Figure 1. Although this finding was initially surprising, familiarity with protein fluorescence suggested that we may, indeed, be measuring Trp in hair under these experimental conditions. This paradigm, indeed, turned out to be correct when we compared the fluorescence emission from the brown hair (above) with authentic L-tryptophan powder under identical experimental conditions, as seen in Figure 2. The complete overlap of the two emission spectra convincingly shows that excitation at this wavelength selectively probes the Trp in hair. It should be mentioned that the unpigmented (white) hair also showed this band, ruling out its association with melanin pigment. An additional feature of the spectrum of damaged hair is the presence of a weak band at ~450 nm. A similar band has also been observed in wool (23) and is probably due to a product of Trp photoxidation.

We have also simulated hair Trp photodamage in a fluorimeter. In this experiment the irradiation wavelength could be precisely selected by using the monochromator of the instrument. Also, since the sample cell remains in the same position at all times, the experimental artifacts associated with reproducibly filling and positioning the cell are eliminated. Piedmont hair was exposed to 295-nm light for various times (see Experimental section for details). The emission spectra, recorded after each irradiation step, are presented in Figure 3. A gradual lowering of the emission intensity in the 338–360-nm range is related to a decrease in the concentration of Trp as a result of photode-

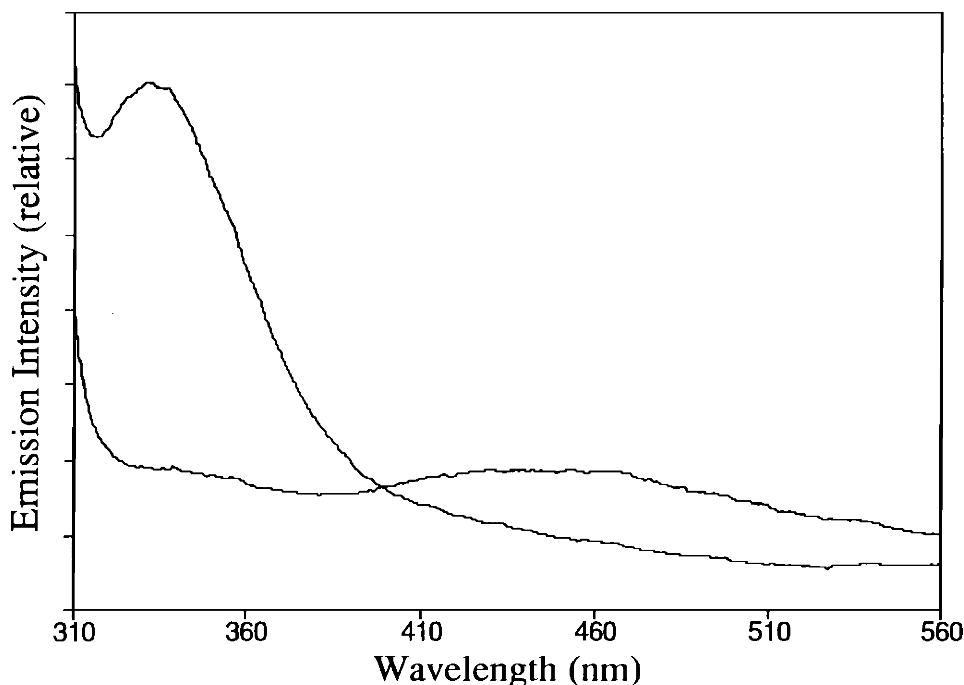

Figure 1. Fluorescence emission spectra of virgin brown (T.J.) and naturally weathered (blond) hair from the same source. The excitation wavelength was 295 nm and the excitation and emission slits were both 5 nm. Notice the loss of ~340 nm band upon hair weathering.

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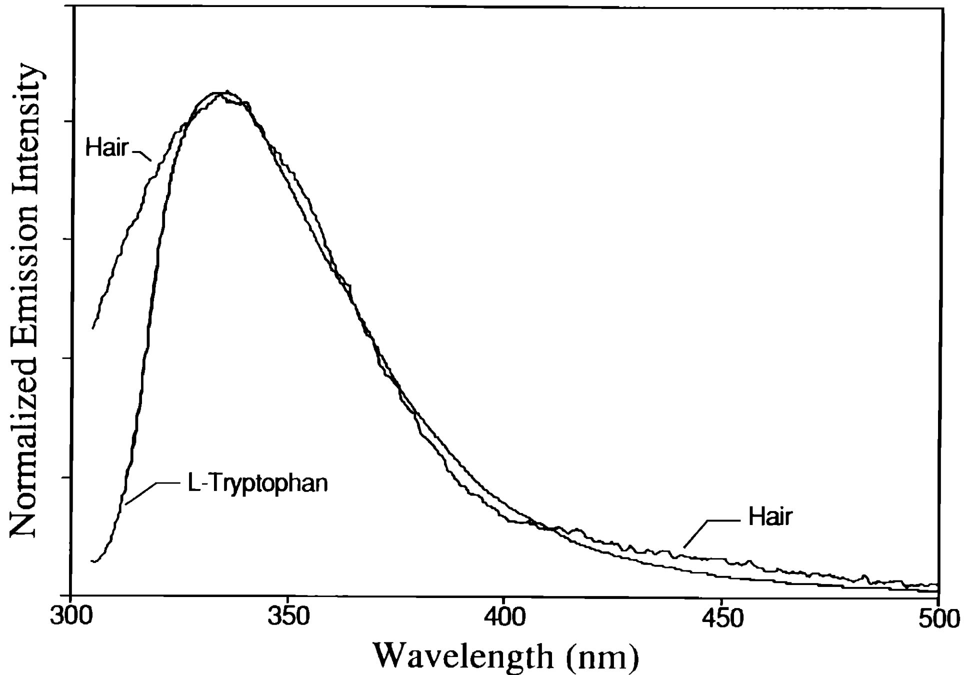

Figure 2. Normalized fluorescence emission spectra of virgin brown hair (noisy spectrum) and solid L-tryptophan (solid line). Excitation wavelength was 295 nm, and the excitation and emission slits were 1 and 5 nm, respectively.

composition. An additional feature of these spectra is the gradual shift of the emission maximum to longer wavelengths as a function of the irradiation time. This might be related to the fact that the observed emission is a superposition of at least two bands with the maxima at 338 nm and 357 nm, as can be demonstrated by spectral subtraction (data not shown). Although Trp fluorescence life-time and, therefore, the emission maximum is known to be sensitive to the polarity of the chromophore environment (24), we are, as yet, unsure of the precise structural significance of this finding.

A similar experiment in which powdered L-tryptophan was exposed to 295-nm light also resulted in a gradual decrease in emission intensity with irradiation time (data not shown), albeit at a different rate and without any shift in the emission maximum. This further confirms that the data obtained for hair under these experimental conditions, indeed, reflect Trp photodamage.

The kinetic experiments of hair photodecomposition performed at various wavelengths in the UVB region indicate that the 295-nm light is most effective in destroying hair Trp, as shown in Figure 4. This most likely results from the fact that this wavelength corresponds to the absorption maximum of Trp.

The kinetics of hair Trp photodamage, as determined by the fluorescence measurements, are complex, and can be affected by a variety of factors. For example, the nonuniformity of exposure of hair protein as a function of distance from the fiber surface to the interior is caused by the attenuation of the exciting (and photodamaging) light beam. Similarly, the intensity of the emitted light is also reduced as it traverses out of the hair. Thus the

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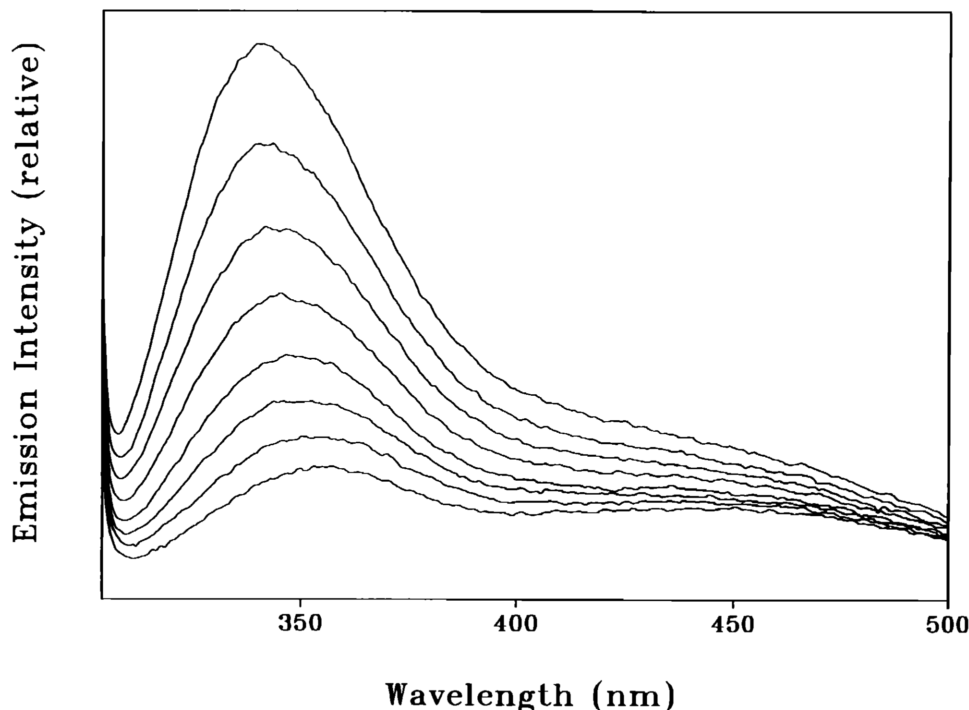

Figure 3. Effect of 295 ± 5 nm irradiation on the tryptophan emission spectrum of Piedmont hair. The excitation wavelength was 295 ± 0.5 nm. The cumulative sample irradiation time for spectra 1–8 from the top was 0, 3, 8, 15, 25, 40, 60, and 105 min, respectively.

surface Trp will be damaged relatively fast, followed by a slower decomposition of Trp inside the fiber, which is exposed to light of lower intensity. The kinetics of photodecomposition of Trp might be further influenced by the differences in the polarity of the local environment of Trp residues (24). In view of these complications, the kinetic data cannot be subjected to rigorous quantitative analysis required to yield mechanistic information. However, the experimental technique is sufficiently precise for comparative purposes.

While hair Trp photodamage can be affected by exposure to artificial light, it was also important to demonstrate the significance of this phenomenon in the context of natural sunlight irradiation. Figure 5 shows the kinetics of Trp photodamage in yak hair upon exposure to sunlight during June 1991 in Stamford, Connecticut. A hair tress was weathered outdoors and the fluorescence measurements were performed at indicated times. The data clearly show that hair Trp damage, indeed, occurs under sunlight exposure. Similar results were obtained for Piedmont (human) hair, suggesting that keratins, in general, are vulnerable to such damage. It should be noted that the conditions of the kinetic experiment of Trp photodamage by natural light cannot be controlled and thus exactly reproduced. The primary reasons for this are the seasonal and time-of-the-day variations in spectral distribution of light, as well as weather patterns such as clouds. For example, the maximum intensity of UV light in Stamford occurs in summer (June and July) and at noontime. Because of the uncertainty in the determi-

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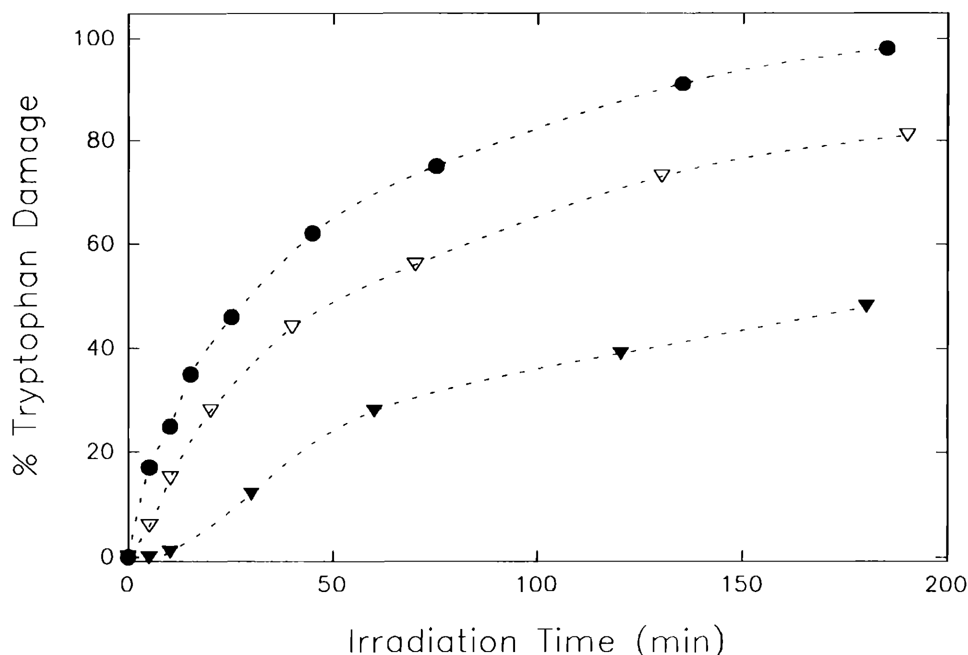

Figure 4. Wavelength dependence of tryptophan destruction. The sample was irradiated in the fluorimeter for the indicated times at either 295 (●), 305 (▽), or 315 nm (▼) and a 10-nm band-pass. Fluorescence emission was then measured by excitation at 295 nm with a nominal 1-nm band-pass. The % tryptophan damage was then calculated from the emission intensities at 340 nm. Note that the sample is assumed to contain 100% tryptophan (0% destruction) prior to laboratory irradiation. Although this is unlikely, it is inconsequential in the present context.

nation of the irradiation dose, quantitative comparisons can be made only for the hair samples subjected to side-by-side weathering. Laboratory experiments with simulated solar irradiation, on the other hand, do not have these limitations and are expected to be fully reproducible.

We have attempted to compare the Trp photodamage rates in the dry and wet fibers, since water is known to play an important role in photolytic reactions. The rate of photodamage was found to be only slightly faster in wet fibers (data not shown). It should, however, be noted that water binds to keratin with high affinity, and it is likely that significant amounts of moisture may have remained in the fibers as a result of sample manipulation at ambient conditions after the drying process. Furthermore, as noted above, the data obtained with dry fibers have a relatively large associated error due to overwhelming background scattering. Further insight into the effect of the medium on Trp photolysis was gained by comparing Trp photodamage rates in hair wetted with water with those in hair saturated with mineral oil. The data, shown in Figure 6, suggest that the hydrophilic, polar medium increases both the rate and extent of Trp photodamage. For example, while 50% measurable hair Trp was lost in eight minutes in the presence of water, it took almost 28 minutes for the same relative damage in the presence of mineral oil. In addition, Trp emission in hydrophobically treated hair was found to be shifted toward shorter wavelength (hypsochromic effect) by about 5 nm,

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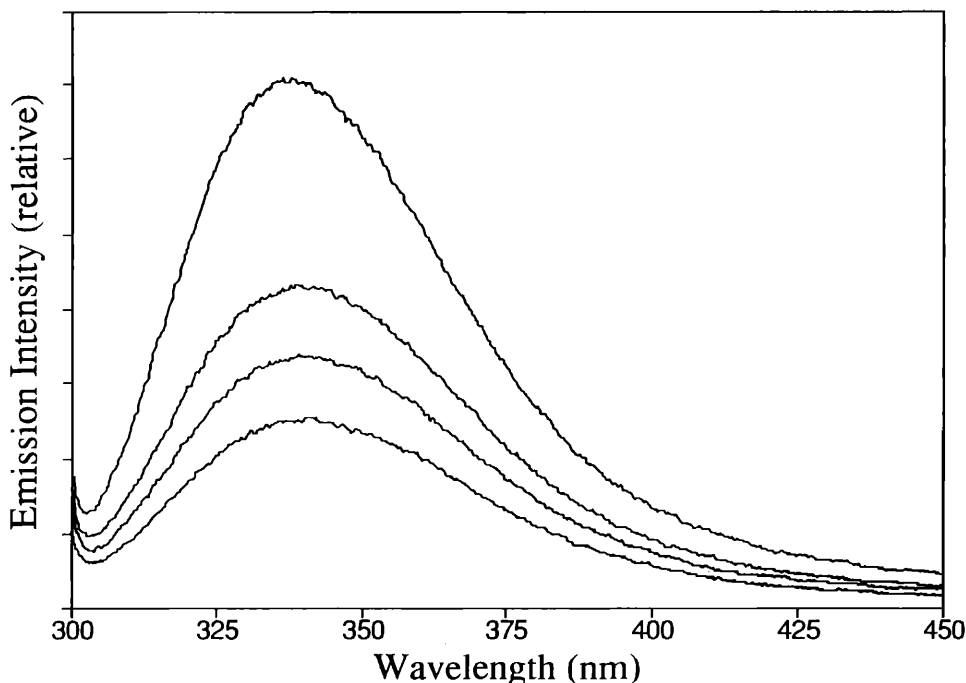

Figure 5. Tryptophan photodamage in yak hair due to solar irradiation during June 1991. The fluorescence was excited at 295 nm, and the excitation and emission slits were 1 and 3 nm, respectively. The spectra from the top represent fluorescence emission after irradiation times of 0, 18 h, 49 h, and 72 h, respectively. It should be noted that both the solar wavelength spectrum, as well as the intensity, show significant day-to-day variation.

compared to that observed for the sample containing water (data not shown). These results are in agreement with the known dependence of the wavelength of emission maximum, as well as the photostability, of Trp on the polarity of its environment (24).

In order to identify other degradative processes associated with weathering besides Trp damage, we have measured IR spectra of pigmented and weathered (blond) hair samples from the same source as in Figure 1. The most significant difference appears to be in the S=O vibration region of the spectra. The data, shown in Figure 7, reveal that weathered hair contains a significantly larger amount of cysteic acid, as judged by the relative intensity of the 1041 cm^{-1} band. This is in agreement with reports in the literature that show that photodamage of both hair and wool results in the oxidation of cystine (disulfide) to cysteic acid (5,25). While weathered hair is characterized by reduced tryptophan and increased cysteic acid, it has not been determined whether these two processes are correlated. It would be very important to establish this relationship in future research, especially in view of the fact that the reduced fiber strength of weathered hair appears to be the consequence of the scission of disulfide bonds and their conversion to cysteic acid residues.

The likely involvement of Trp damage with other aspects of hair photodamage can be inferred from the existing literature on photochemistry of proteins and amino acids (9). It has been shown that besides being photolabile, Trp sensitizes the photolysis of other chromophoric and non-chromophoric amino acids. Using synthetic peptide hormones,

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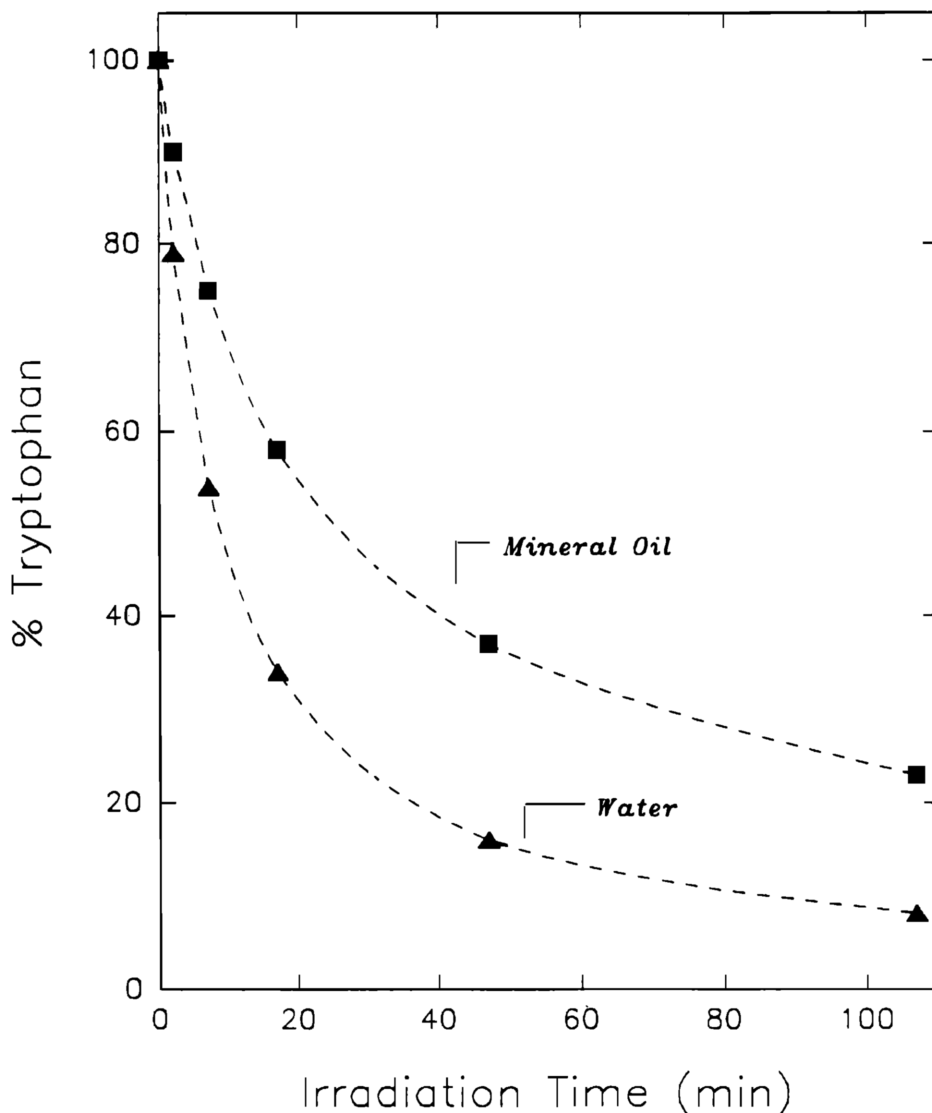

Figure 6. Amount of tryptophan photodestruction in hair upon irradiation at 295 ± 5 nm for the indicated times. The hair samples were either soaked in water or in mineral oil. The % tryptophan damage was calculated as explained in the caption to Figure 4. Note that the half-life (50% destruction time) in an aqueous environment is almost thrice that in mineral oil.

it has been shown that basic amino acids flanking Trp in the primary sequence undergo Trp-sensitized photodamage (26–28). Electron transfer from an excited Trp to a neighboring Tyr in model peptides has been documented (29). Literature reports also suggest Trp-sensitized damage to the disulfide bond (30). It should be noted here that although the disulfide bond itself has a much weaker absorption in the UVB as compared to Trp (ϵ_{290} = 40 and 4500 M^{-1} cm^{-1}, respectively) (9,11), the net absorption, due to the high disulfide concentration in keratins, may in fact be non-negligible. Preliminary experiments (data not shown) using FTIR microscopy, which is very sensitive in de-

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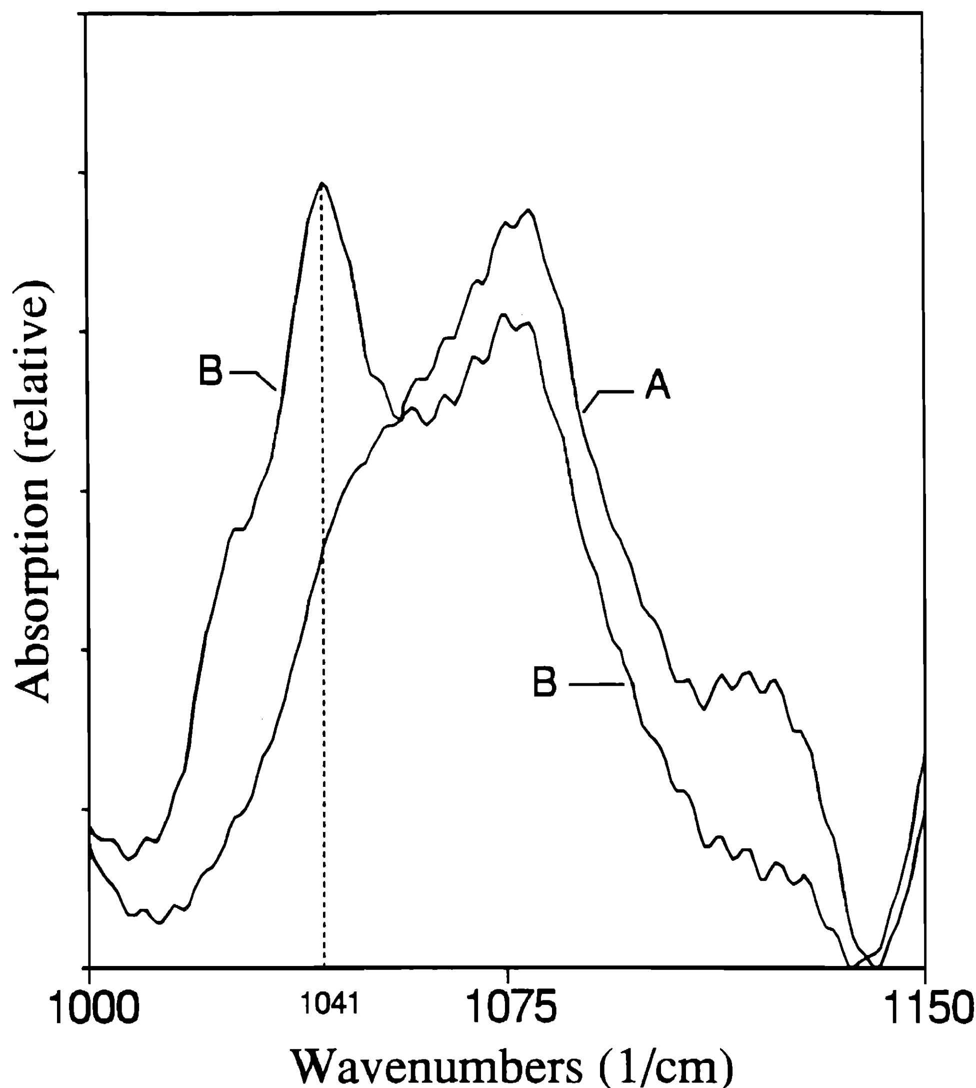

Figure 7. FTIR absorption spectra of virgin brown (T.J.) and naturally weathered (blond) hair from the same source. The spectra were measured in the IR-Plan™ microscope. The data were flattened with a curved baseline function between 1368, 1142, and 1000 cm^{-1} and enhanced with a 13-point smoothing routine.

tecting disulfide oxidation products, however, did not reveal any oxidation under the irradiation conditions that resulted in significant Trp loss in hair. Tryptophan photodamage, therefore, appears to kinetically precede disulfide photoxidation.

The strength and structural stability of hair is essentially governed by the integrity of its protein constituents. Besides the all-pervasive peptide bonds, the protein stability also derives from the amino acid side-chain interactions. These include the electrostatic forces between basic and acidic amino acids (salt linkages), the hydrophobic interactions between the aromatic and alkyl side groups, the hydrogen bonding, and the isopeptidic interactions between various groups in the protein structure. In cystine-containing

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proteins such as keratin, the disulfide bond contributes strongly to the overall stability and integrity. Trp residue, besides being the most hydrophobic amino acid, can also participate in hydrogen bonding through the N-1 proton in the indole ring. The photodamage to Trp will, therefore, directly affect the stability of hair protein. It seems also very likely that Trp photodamage may not be just a local casualty, but may represent the earliest event in the photodamage process involving other residues, ultimately resulting in the observed deterioration of the hair condition. So far, there is fairly strong evidence linking Trp photodecomposition to yellowing of wool fibers (31). No other direct correlation between the Trp loss in hair and changes in other observables has been shown so far.

PREVENTION OF PHOTODAMAGE

An obvious application of these studies is in preventing, or at least reducing, hair photodamage. There are two main strategies. The first is to reduce the intensity of incident light by using photofilters. This can be accomplished by coating hair with suitable sunscreens characterized by absorption spectra overlapping the Trp absorption band. The UVB sunscreen molecules typically have extinction coefficients approximately 60 times higher than that of Trp at 300 nm (9,32). The second approach would be to eliminate, or slow down, the photodecomposition through intervention in the photochemical pathway. This may involve changing the Trp microenvironment by using solvents that would retard the excited-state decomposition, or a somewhat complicated step such as treatment with non-chromophoric materials that would quench the photochemical intermediates. The non-chromophoric photoprotection route, although extremely promising, remains to be explored since it requires a detailed understanding of the mechanism of the photochemical reactions in hair keratin.

As a starting point, we have evaluated the photoprotective ability of hair care formulations containing common UVB sunscreens. Due to the spectral overlap of such compounds with the Trp absorption spectrum, they are likely to filter off a significant fraction of the incident light. For example, we have calculated that ~0.1 μ film of a formulation containing 0.4% sunscreen ($\epsilon \sim 20{,}000\ M^{-1}\ cm^{-1}$), out of a total of ~4% solids, would filter out ~20% incident light at its λ_{max} (absorption maximum). These calculations, however, do not take into account the photodecomposition of the sunscreen itself. It is thus probable that the incident light would be initially significantly attenuated, with the photofiltering effect diminishing as a function of the irradiation time as the sunscreen molecules are themselves gradually sacrificed.

The experimental results obtained with a prototype leave-in hair treatment containing 0.2% octylmethoxy cinnamate are shown in Figure 8. The sample and the control were irradiated in a solar simulator. It is evident from these data that the product reduces the extent of Trp damage by about 40%. Similar experiments were performed for a variety of other cosmetic formulations such as fixatives, conditioners, and shampoos. We conclude that consequential surface deposition of sunscreens, and photodamage protection of the order of tens of percent, can only be obtained with leave-in products. On the other hand, the presence of conventional sunscreen in shampoos does not produce measurable photoprotection if the treatment is performed in the usual manner, which includes short-term (30–60 s) lather followed by rinsing. This is not surprising since (i) compounds such as DEA methoxycinnamate or octyl methoxycinnamate do not possess

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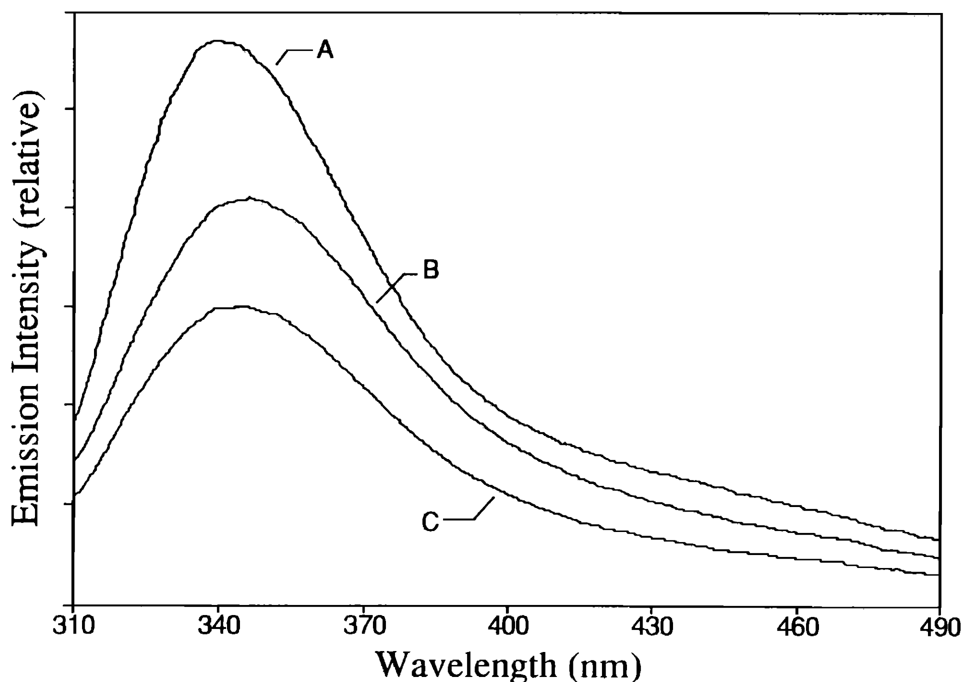

Figure 8. Hair tryptophan photoprotection afforded by hairspray containing 0.2% octylmethoxycinnamate. The treated and the control samples were irradiated in a solar simulator for a total of 30 h. The sample was irradiated for 10 h each time, after which it was shampooed and retreated. The upper curve is due to unirradiated control, the middle curve is the treated sample, and the lowest curve is the irradiated control. The difference between the last two curves is the absolute photoprotection due to the treatment. The excitation was at 295 nm, and the excitation and the emission slits were 1 and 5 nm, respectively.

strong affinity toward the hair surface, (ii) the contact time of the formulation with hair is too short to allow the penetration of sunscreen inside the fiber, and (iii) the application of the treatment is followed by rinsing, which removes weakly adsorbed species from the fiber. In contrast to this, measurable photoprotection can be imparted to hair soaked for two hours in a shampoo containing octyl-PABA and rinsed with water, while similar treatment in the absence of sunscreen has virtually no effect. This experiment (data not presented), although unrealistic from the point of view of practical application, nevertheless confirms the notion that insufficient sunscreen deposition is the primary reason for the ineffectiveness in photoprotection of rinse-off type compositions. Formulating with UV absorbers characterized by high affinity to hair, brought about by incorporation of cationic, hydrophobic, or reactive functions into the structure of a sunscreen, might enhance the performance of such treatments.

CONCLUSIONS

It has been shown that fluorescence spectroscopy can be used to monitor the decomposition of tryptophan by exposure to natural and artificial UV light. The application of light absorbers to the surface of hair was shown to significantly slow down the process of Trp destruction. In addition to this, the rate of tryptophan photodamage can be increased by absorption of water, which creates a polar environment surrounding Trp

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residues, or reduced by treatment with mineral oil, which is nonpolar and hydrophobic. Although direct correlations are presently missing, we speculate that Trp disappearance may be the first, deleterious, aspect of hair photodamage, initiating a cascade of events leading to the breakage of other amino acids, particularly those containing disulfide bonds. An apparent consequence of these reactions might be the reduction of the mechanical strength of hair, loss of fiber integrity, loss of luster, and increased swelling. The developed methodology can be used to exploit the photolability of intrinsic hair tryptophan in order to test and optimize hair care formulations designed to provide photoprotection.

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