International Journal of Cosmetic Science

International Journal of Cosmetic Science, 2013, **35**, 539–545 doi: 10.1111/ics.12054

Towards an insight on photodamage in hair fibre by UV-light: An experimental and theoretical study

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Received 06 March 2013, Accepted 20 April 2013

Keywords: *ab initio* calculation, hair fibre, kynurenine, N-formylkynurenine, photodegradation, tryptophan

Synopsis

OBJECTIVES: In this research, an experimental and theoretical study was conducted to design a photodegradation mechanism of the amino acid tryptophan (Trp) in hair fibres.

METHODS: For the experimental research, Caucasian hair fibres were exposed to several different solar radiation simulation periods. Then, Trp and its photoproducts (N-formylkynurenine and kynurenine) were assayed by excitation and emission spectroscopic analysis.

RESULTS: For the theoretical study, reactions involved in the photodegradation of Trp were evaluated by high-level quantum mechanical calculations in a density functional theory (DFT) framework which indicate a probable Trp degradation mechanism with a minimum expended energy pathway.

CONCLUSION: The biochemistry concerning these reactions is essentially important for a biological system where the degradation of Trp occurs.

Résumé

OBJECTIFS: Une étude expérimentale et théorique a été menée pour concevoir un mécanisme de photo-dégradation de l'acide aminé tryptophane (Trp) dans les fibres capillaires.

METHODES: Pour la recherche expérimentale, des fibres de cheveux caucasiens ont été exposés à plusieurs différentes périodes de simulation du rayonnement solaire. Puis Trp et ses photo-produits (N-formylkynurenine et kynurenine) ont été analysés par l'analyse spectroscopique d'excitation et d'émission.

RESULTATS: Pour les études théoriques, les réactions impliquées dans la photo-dégradation de Trp ont été évaluées par des calculs de mécanique quantique de haut niveau dans un cadre de la théorie de la densité fonctionnelle (DFT) qui indiquent un mécanisme de dégradation probable de Trp, avec une voie minimale d'énergie dépensée.

CONCLUSION: La biochimie de ces réactions est d'une importance essentielle pour un système biologique dans lequel la dégradation de Trp se produit.

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Introduction

Hair fibre is a natural polymer formed by a fibrous structure of proteins which belong to the keratin family. Morphologically, the structure has two distinct structures: an external layer called cuticle and an internal mass called cortex. The cortex consists of elongated cells oriented parallel to the fibre axial axis. These cells contain microfibres formed by a tridimensional crystalline keratin helix and are embedded on an amorphous matrix which is rich in cystine. A small amount of the total fibre mass can be attributed to melanin pigments and lipids [1–3].

Ultraviolet and visible radiation damages hair [4–9], but little is known about biochemical and photochemical changes caused by radiation exposure. The mechanisms of photodegradation are still unexplained, and there is a lack of scientific studies regarding hair photodamage [10, 11]. Sun radiation on hair causes dryness, reduced strength, a rough surface texture, loss of colour, decreased lustre, stiffness, brittleness and an overall dull, unhealthy appearance. Although it is less important than the study of skin photodamage, from the point of view of health risks, healthy hair is associated not only with beauty, but also with overall self-esteem [12].

The photochemical degradation and photoyellowing of wool, a natural fibre that at various levels shows a series of features similar to human hair has been studied extensively [13–19]. The melanins, which are hair and wool pigments [20], provide some photochemical protection for hair proteins, especially at lower wavelengths where both the pigments and the proteins absorb light [21] by absorbing and filtering the impinging radiation and subsequently dissipating this energy as heat. Their high absorption capacity can be explained in terms of their extensive system of conjugate carbonyl groups and double bonds which captures a large fraction of the radiation and immobilizes many of the free radicals formed upon the absorption of the UV radiation photosensitive amino acids in hair and prevents the transport of these free radicals into the keratin matrix [22]. However, in the process of protecting hair proteins from light, the pigments are degraded or bleached.

As in all photoprocesses, the essential element in hair photodamage is light absorption by the fibre, but this process is significant only if the protein absorbs the incident light. For most proteins without bound (covalent or non-covalent) cofactors or prosthetic groups, this phenomenon occurs only with light ($\lambda \leq$ ca. 320 nm) [23].

The most significant chromophores in proteins that absorb in the UVB region (280–320 nm) are the amino acids tyrosine (Tyr,

λ_{max} = 275 nm, ϵ_{290} = 100 M^{-1} cm^{-1}), tryptophan (Trp, λ_{max} = 280 nm, ϵ_{290} = 4500 M^{-1} cm^{-1}) and the disulphide bonds of cystine (λ_{max} < 200 nm, ϵ_{290} = 40 M^{-1} cm^{-1}); UV spectra of these amino acids are available in Ref. [24]. The longer wavelength UVA and visible light are unlikely to cause direct damage as they are not absorbed by proteins [25, 26]. The absorption of UVB light by Trp, Tyr and cystine can give both excited state species and radicals via photoionization [24, 27]. The relative energies of the short-lived first excited singlet states decrease the order: Tyr > Trp [27] and can result in rapid energy transfer from Tyr to Trp which is why the fluorescence spectra of most proteins are often dominated by Trp spectra.

For Trp a fluorescent, monoexponential decay was observed. The conversion of the α-carboxyl group into the corresponding amide or its protonation results in a complex fluorescence decay [28]. Some explanations for this behaviour were established by Gauduchon *et al.* [29] in a ground-state rotamer model. Cowgill [30, 31], Tournon *et al.* [32] and Feitelson [33] suggested that the occurrence of luminescence by the aromatic amino acids chain is due to a transfer of charges between the excited aromatic chromophore (phenol ring) acting as a donor and the electrophilic units in the amino acid backbone (the carbonyl of the amide group) acting as an acceptor.

Schafer *et al.* [34] studied Trp photodegradation and the photodegradation products (N-formylkynurenine, kynurenine, 3-Hydroxykynurenine) by high performance liquid chromatography (HPLC). They concluded that these photoproducts of Trp contributed to the photoyellowing of wool.

In hair, the estimated content for the Trp ranges from 0.2% to 1.0% [3, 35], but this amino acid plays a significant role in the photochemistry of keratins and proteins in general (as discussed above). In this respect, Pande and Jachowicz [7] monitored the photodegradation of hair by a spectroscopic technique as a function of the relative concentration of Trp. They also demonstrated that hair care formulations containing sunscreens can reduce the extent of photodamage.

Robbins and Bahl [36] examined the effects of UV on cystine by chemical radiation on disulphide sulphurs in hair via electron spectroscopy for chemical analysis (ESCA). They suggest that for photochemical degradation, the C-S fission route is recommended.

Cystine is a quenching agent for Tyr; previous research [37] has demonstrated that the quenching mechanism occurs via an electron transfer process with the formation of the radical anion $RSSR^{\cdot -}$ Although the excited Trp undergoes intersystem crossing to triplet states, studies on wool have suggested that singlet states are quenched more effectively than triplet states [38]. However, there is no evidence of an energy transfer from Trp to cystine in an aqueous system during irradiation [34].

This paper reports studies on Caucasian hair fibres which were exposed to several different solar radiation simulation periods; then, Trp and its photoproducts were evaluated by excitation and emission spectroscopy analysis. A Trp photodegradation process by high-level quantum *ab initio* mechanical calculations is proposed. Rather than elucidate, every possible ways or intermediate steps that this degradation can undergo, our aim is to contribute a valid way of degradation.

Experimental section

Materials

Untreated black Caucasian hair was obtained from De Meo Brothers, New York, U.S.A. Twenty-one (21) hair tresses weighing approximately 5.0 g with a length of 25.0 cm each were prepared for this work. The hair samples were pre-treated with a 3% aqueous solution of lauryl ammonium sulphate (LAS) and then dried in a standard environment at 55 ± 5% relative humidity and 22 ± 2°C for 24 h prior to the experimental procedures.

Exposition of solar radiation

Hair tresses were exposed to simulated solar radiation in Atlas Weather-Ometer (Model 65 XW-WR1) equipment with a xenon light source of 6500 W. The total radiation was 0.35 W m^{-2} at 340 nm, and the humidity and temperature conditions of the chamber in the equipment were controlled according the ASTM G 155 norm. The emission spectrum is closer to the entire solar spectrum and the ageing time approximately 8.7 times major than the natural ageing solar time. The samples were simultaneous exposed in groups of three tresses: T0 – control, T24 – 24 h, T48 – 48 h, T96 – 96 h, T144 – 144 h, T192 – 192 h and T240 – 240 h.

Fluorescence measurements

Fluorescence evaluations were conducted with a spectrophotometer Fluorolog – Jobin Yvon Horib (Model number FL3-12) with a xenon light source which provided the full solar spectrum.

The excitation line utilized for the Trp was 294 nm (absorbance) which has the highest emission (fluorescence) intensity of ~340 nm. The excitation line utilized for the N-formylkynurenine was 320 nm with an emission of ~420 nm. The excitation line utilized for the kynurenine was 360 nm with an emission of ~435 nm.

Hair tresses were placed on a sampler at a 45° angle to the photomultiplier cell. A total of three spectroscopic analyses were completed for each group of three tresses (n_{total} = 9, $n_{independent}$ = 3). Basal and post-exposure measures were conducted.

Computational method

Calculations were carried out with the GAUSSIAN 98 program [39]. The simulations were performed by using the density functional theory (DFT) at the unrestricted B3LYP level [40, 41] using standard all-electron 6–31++G** [42] basis sets for H, C, O and N atoms. The inclusion of polarized and diffuse functions and correlated methods is mandatory in the description of atoms in the amino group [43]. Researchers use this level and basis set because it is applicable for calculations on systems involving amino acids and nucleic basis sets [44–46]. Quantum mechanical computations can provide invaluable support for experimental data because many molecules that have not been experimentally measured can be evaluated. Full geometry optimizations in the gas phase have been carried out for all stationary points. Optimized structure harmonic frequencies were calculated to confirm the nature of the minima (zero negative eigenvalues of the Hessian matrix).

Results and discussion

Figure 1a shows normalized Trp emission spectra for hair tresses exposed to 24, 48, 96, 144, 192 and 240 h of solar radiation and control. Each spectrum corresponds to three tresses with tripled readings. The excitation length for the Caucasian fibres was 294 nm, and the most intense emission was at approximately 340 nm. The humidity control was rigorous as the Trp photoluminescence (PL) is quite sensitive to moisture variations. Somers and

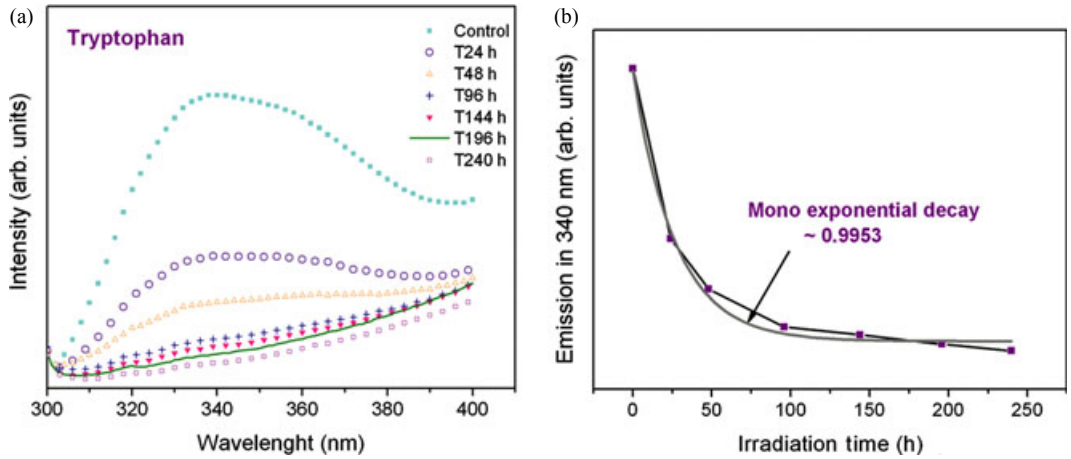

Figure 1 (a) Normalized photoluminescence emission spectra of Trp for Caucasian hair fibres exposed to solar radiation at 24, 48, 96, 144, 192, 240 h plus the control. Excitation energy of 294 nm emission ~340 nm; (b) Trp decomposition as a function of irradiation time and monoexponential decay fitting.

Ceulemans [47] studied the conformational changes in the Trp molecule when hydrated with water. The Trp degradation kinetics is also altered as a function of the amount of moisture because photolytic processes are usually accelerated in water. Therefore, all hair tresses were exposed to radiation under similar conditions of approximately 50 ± 5% moisture. Figure 1b shows the maximum intensity of the Trp emission as a function of increased exposure time.

The Trp degradation kinetic determined by fluorescence measurements is complex and might be affected by various factors, for example, the non-uniform exposure of fibres as a function of their thickness will cause more damage to their surface than to their interior which results in a higher degradation on the surface. As the fibre hair is a bio-organic material, there is a variability, in the triplicate measurements for each group o tresses (around 7%). Degradation kinetics will also be influenced by polarity changes in the environment due of Trp residues to formation after its decomposition. Furthermore, the photon beam is not lined, and therefore, the photomultiplier cell does not detect all the emission energy. Due to these complexities, a rigorous thermodynamic and quantitative analysis is impossible. However, as can be observed in Fig. 1b, there is a very close approximation of the curve with a monoexponential decay (approximation error of ~0.0047). Spectroscopic measurements also suggest that after 96 h of exposure, most of the Trp in the fibre has been degraded. The same spectroscopic technique used for the Trp degradetion investigation was used to acompanied the Trp residues formation.

The cleavage of the Trp indole ring facilitates the formation of N-formylkynurenine which can decay after reduction of the formyl group to kynurenine [34]. The usual excitation line for the luminescent detection of N-formylkynurenine and kynurenine is 325 nm and 365 nm, respectively [34].

In hair, the excitation wavelength that results in the N-formylkynurenine higher intensity emission is 320 nm with the highest emission peak centred at approximately 420 nm. Figure 2a illustrates normalized emission spectra for N-formylkynurenine formation as a function of radiation exposure time. Figure 2b elucidates this behaviour at the wavelength with highest emission intensity. The exponential growth fitting of this subproduct formation shows an experimental approximation of 0.0125 and can be related to Trp monoexponential decay. In fact, Trp degradation can provide other subproducts [23], but with the Trp growth degradation, there is an increased formation of N-formylkynurenine.

Kynurenine is next in the sequence of residues of Trp residues. In hair, excitation energy at 350 nm yielded the highest emissions at approximately 435 nm. A similar behaviour of N-formylkynurenine was observed in kynurenine (see Fig. 3a); however, the emission intensity was much higher. Figure 3b illustrates kynurenine behaviour under the highest emission wavelength as a function of radiation exposure time. The exponential growth fitting of this subproduct formation shows an experimental approximation of 0.0257 which can be related to Trp monoexponential decay.

N-formylkynurenine and kynurenine are significant photosensitizing agents and much better O_2 generators than Trp itself [48–50]. Thus, the formation of N-formylkynurenine and kynurenine on proteins or peptides may result in further enhancement of the extent of photooxidation of the target protein in a long UV exposure time trough either through the generation of further O_2 or possibly via sensitization of the side-chain peroxides decomposition. Thus, for Trp, the (relatively) poor sensitizing activity and high physical quenching activity (compared to a chemical reaction) may play a role in the initial protection of human hair against UV damage; however, after a high level of exposure, the gradual accumulation of the more potent sensitizers N-formylkynurenine and kynurenine in the photodamaged hair may result in damage enhancement.

Direct photooxidation mechanisms arising from the absorption of UV radiation by the protein structure (primarily side-chains) or bound chromophores which generate an excited state or radicals as a result of photoionization are often referred to as a Type 1 process [23] which were carefully analysed in this study.

The *ab initio* reaction calculations involving Trp decomposition with light and oxygen were conducted. Considering that most of the biological reactions occur in H_2O presence, this molecule was added in reactions which were studied. All stationary points of potential surface energy were fully optimized. Figure 4 show pathways suggested for the Trp decomposition reaction.

Light ($h\nu$) excites the Trp molecule (A) in hair fibres which form a free radical of Tryptophan (TrpH*) (B) (A B). This step is still unclear in the literature, but the necessary energy for the forma-

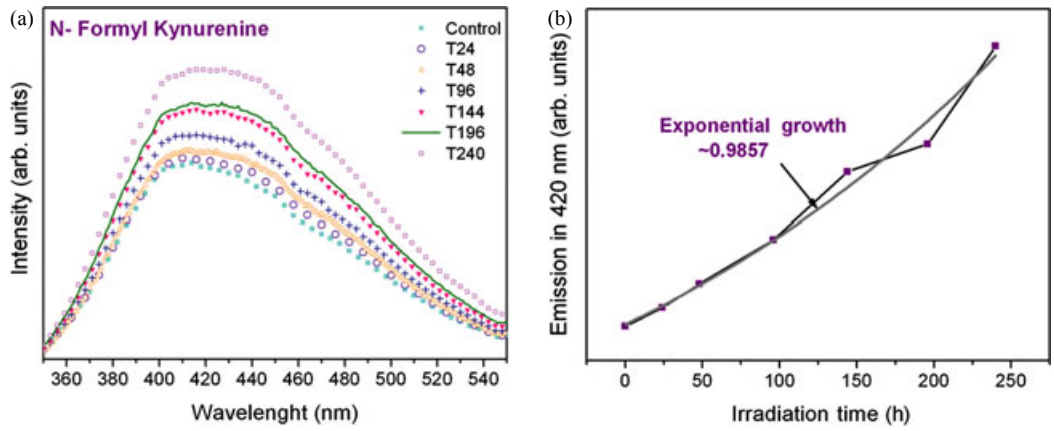

Figure 2 (a) Normalized photoluminescence emission spectra of N-formylkynurenine formation for Caucasian hair fibres exposed to solar radiation at 24, 48, 96, 144, 192, 240 h plus the control. Excitation energy of 320 nm, maximum emission ~420 nm; (b) N-formylkynurenine formation with increased time of exposition and monoexponential growth fitting.

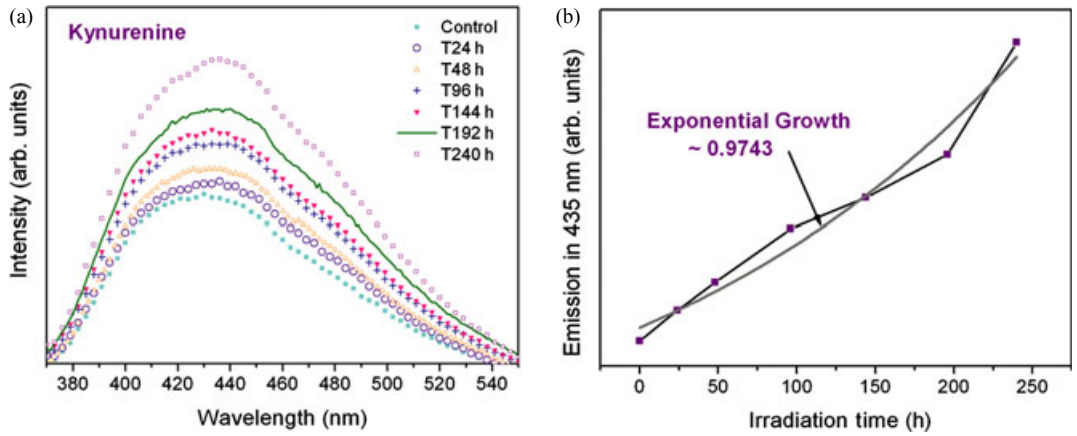

Figure 3 (a) Normalized photoluminescence emission spectra of the formation of kynurenine formation for Caucasian hair fibres exposed to solar radiation at 24, 48, 96, 144, 192, 240 h plus the control. Excitation energy of 360 nm, maximum emission ~435 nm; (b) kynurenine formation with increased time of exposition and monoexponential growth fitting.

tion of this radical in the presence of light and H_2O was +10.27 eV. The oxygen in the environment in its ground state is excited in TrpH*, as it has one uncoupled pi (π) electron. Therefore, oxygen can behave as a dienophile capable of producing a Diels–Alder-type reaction with the TrpH* radical (B). This reaction is commonly referred to as an addition cycle which requires a combination in a system where two π two electrons participate. At this point, one π electron from the Trp pyrrole ring and the one π electron from oxygen results in a (C) (B C) molecule. In this step, an additional +2.19 eV is necessary for the reaction to occur. In the next step of this mechanism, a peroxide (D) is formed with the pyrrole ring by the addition of one H^+ of H_3O^+ of the environment (C D). The reaction is spontaneous with a −12.29 eV energy liberation. Under the presence of light, this new compound is unstable, and the peroxide (D) decomposes to N-formylkynurenine (E) (D E). This step is spontaneous and emits −3.81 eV of energy. Finally, the light continues the photolysis process which results in kynurenine formation (F) (E F) that occurs in other photolyses. This process is not spontaneous and requires +0.42 eV of energy, that is, in the absence of light, N-formylkynurenine (E) is a more stable molecule than the kynurenine (F) molecule.

Figure 5 shows the potential energy variation (ΔE) of reaction steps as well as two mechanisms for the reaction: Mechanism I is composed of steps: A B C D E F, and Mechanism II shows the steps which take place for A D E F.

In Mechanism I, +12.46 eV of energy is spent in changes from A to B and B to C. In this case, the direct passage from A to D is the most probable step where the reaction occurs (Mechanism II). Molecules B and C are stable as the *ab intio* calculations indicate, but the pathway to their existence is too expensive energy wise. Mechanism II, where two oxygens are added to the A (Trp) molecule, requires +0.17 eV of energy and is the most probable pathway for the reaction to occur. In both Mechanism I and II, the energetic balance of the reaction is constituted by the liberation of −3.22 eV.

Observing the Trp photodegradation is difficult since the kinetics is very fast. Our *ab initio* calculations elucidate photochemical

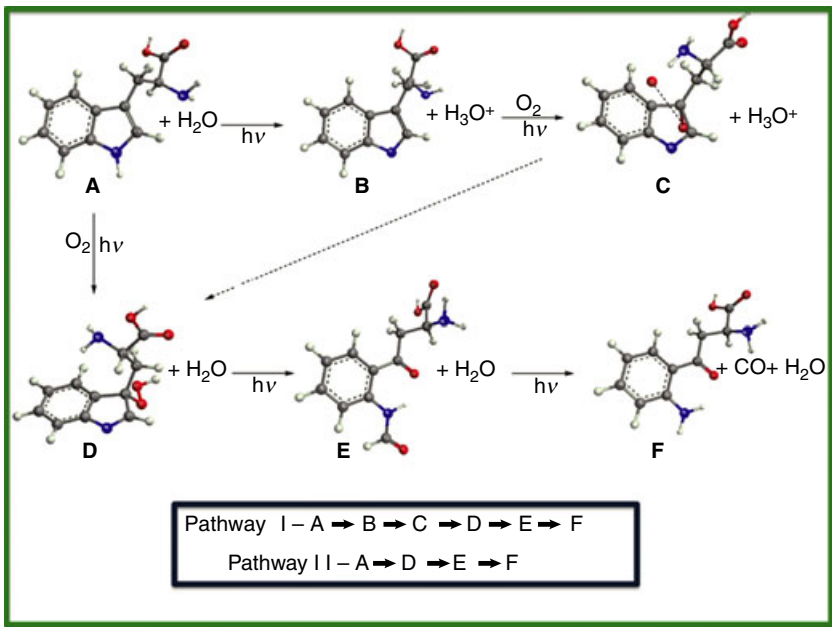

Figure 4 Proposed *ab initio* calculated reaction for Trp degradation.

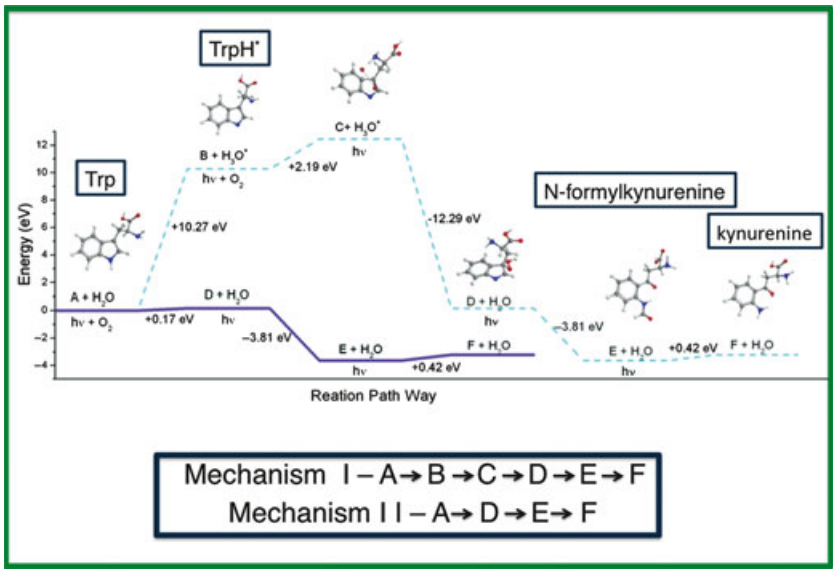

Figure 5 Potential energy diagram of the Trp degradation reaction where two mechanisms (I or II) can occur.

pathways involved and are useful to understand their role in bio-processes.

Conclusions

The Trp degradation kinetics as determined by fluorescence measurements is complex and may be affected by various factors. However, fluorescence is a molecular level technique and could become a valuable instrument for the characterization of hair fibres after exposure to large amounts of photonic radiation. In this manner, this experimental technique is sufficiently precise for comparative and qualitative purposes.

The Trp degradation produces free radicals and two residues, and the kinetic evolution of the formation of these residues has shown that they increase in concentration as a function of radiation exposure time.

The *ab initio* calculation for Trp degradation reactions steps was carefully analysed, and a mechanism (Mechanism II) was proposed

as the most probable pathway for the reaction where a minimum expended energy pathway occurs.

This technique can be applied to the study of advanced cosmetic photoprotector products and in other areas of biochemistry where the reactions of Trp photodegradation occur.

Acknowledgements

The authors gratefully acknowledge the financial support of the Brazilian agencies FAPESP, CNPq and CAPES.

References

1. Jachowicz, J. Hair damage and attempts to its repair. *J. Soc. Cosmet. Chem.* **38**, 263–286 (1987).
2. Robins, C. *Chemical and Physical Behaviour of Human Hair*. Springer-Verlag, New York (1988).
3. Zviak, C. and Dawber, P.R. Chap. 1. Hair Structure, Function, and Physiochemical Properties. In: *Hair Structure and Function*. (Zviak, C., ed.), pp. 23–31. M. Dekker, New York (1989).
4. Nogueira, A.C.S. and Joekes, I. Hair color changes and protein damage caused by ultraviolet radiation. *J. Photochem. Photobiol. B-Biol.* **74**, 109–117 (2004).
5. Millington, K.R. and Church, J.S. The photodegradation of wool keratin .2. Proposed mechanisms involving cystine. *J. Photochem. Photobiol. B-Biol.* **39**, 204–212 (1997).
6. Ratnapandian, S., Warner, S.B. and Kamath, Y.K. Photodegradation of human hair. *J. Cosmet. Sci.* **49**, 309–320 (1998).
7. Pande, C.M. and Jachowicz, J. Hair photodamage – measurement and prevention. *J. Soc. Cosmet. Chem.* **44**, 109–122 (1993).
8. Monteiro, V.F., Pinheiro, A.S., Leite, E.R., Agnelli, J.A.M., Pereira-Da-Silva, M.A. and Longo, E. UV radiation: aggressive agent to the hair – AFM, a new methodology of evaluation. *J. Cosmet. Sci.* **54**, 271–281 (2003).
9. Rele, A.S. and Mohile, R.B. Effect of mineral oil, sunflower oil, and coconut oil on prevention of hair damage. *J. Cosmet. Sci.* **54**, 175–192 (2003).
10. Beyak, R., Kass, G.S. and Meyer, C.F. Elasticity and tensile properties of human hair .2. Light radiation effects. *J. Soc. Cosmet. Chem.* **22**, 667–678 (1971).
11. Wolfram, L. *The Reactivity of Human Hair: A Review (Hair Research)*. Springer-Verlag, New York (1981).
12. Nogueira, A.C.S., Dicelio, L.E. and Joekes, I. About photo-damage of human hair. *Photochem. Photobiol. Sci.* **5**, 165–169 (2006).
13. Graham, D.R. and Statham, K.W. The Photochemical oxidation of wool in the presence of fluorescent compounds. *J. Soc. Dye. Colour.* **72**, 434–438 (1956).
14. Graham, D.R. and Statham, K.W. Tryptophan content of wool. *Nature* **181**, 861 (1958).
15. Graham, D.R. and Statham, K.W. Effect of temperature on the felting and dyeing properties of wool. *J. Soc. Dye. Colour.* **75**, 386–387 (1959).
16. Lennox, F.G. and Rowlands, R.J. Photochemical degradation of keratins. *Photochem. Photobiol.* **9**, 359–367 (1969).
17. Smith, G.J., Thorpe, M.R., Melhuish, W.H. and Beddard, G.S. Fluorescence of tryptophan in keratin. *Photochem. Photobiol.* **32**, 715–718 (1980).
18. Collins, S., Davidson, S., Greaves, P.H., Healey, M. and Lewis, D.M. The natural fluorescence of wool. *J. Soc. Dye. Colour.* **104**, 348–352 (1988).
19. Millington, K.R. Photoyellowing of wool. Part 1: factors affecting photoyellowing and experimental techniques. *Color. Technol.* **122**, 169–186 (2006).
20. Ozeki, H., Ito, S. and Wakamatsu, K. Chemical characterization of melanins in sheep wool and human hair. *Pigment Cell Res.* **9**, 51–57 (1996).
21. Hoting, E., Zimmermann, M. and Hocker, H. Photochemical alterations in human hair. 2. Analysis of melanin. *J. Soc. Cosmet. Chem.* **46**, 181–190 (1995).
22. Tolgyesi, E. Weathering of hair. *Cosmet. Toilet.* **98**, 29–33 (1983).
23. Davies, M.J. and Truscott, R.J.W. Photo-oxidation of proteins and its role in cataractogenesis. *J. Photochem. Photobiol. B-Biol.* **63**, 114–125 (2001).
24. Bensasson, R.V., Land, E.J. and Truscott, T.G. *Pulse Radiolysis and Flash Photolysis: Contributions to the Chemistry of Biology and Medicine*. Pergamon Press, Oxford (1983).
25. Borkman, R.F. Uv action spectrum for tryptophan destruction in aqueous-solution. *Photochem. Photobiol.* **26**, 163–166 (1977).
26. Tallmadge, D.H. and Borkman, R.F. The rates of photolysis of the 4 individual tryptophan residues in Uv exposed calf gamma-2 crystallin. *Photochem. Photobiol.* **51**, 363–368 (1990).
27. Bensasson, R.V., Land, E.J. and Truscott, T.G. *Excited States and Free Radicals in Biology and Medicine*. Oxford University Press, Oxford (1993).
28. Ross, J.B.A., Laws, W.R., Sutherland, J.C., Buku, A., Katsoyannis, P.G., Schwartz, I.L. and Wyssbrod, H.R. Linked-function analysis of fluorescence decay kinetics – resolution of side-chain rotamer populations of a single aromatic amino-acid in small polypeptides. *Photochem. Photobiol.* **44**, 365–370 (1986).
29. Gauduchon, P. and Wahl, P. Pulse fluorimetry of tyrosyl peptides. *Biophys. Chem.* **8**, 87–104 (1978).
30. Cowgill, R.W. Fluorescence and protein structure.X. Reappraisal of solvent and structural effects. *Biochim. Biophys. Acta* **133**, 6–18 (1967).
31. Cowgill, R.W. Fluorescence and structure of proteins .2. Fluorescence of peptides containing tryptophan or tyrosine. *Biochim. Biophys. Acta* **75**, 272–273 (1963).
32. Tournon, J., Elbayoum, M.A. and Kuntz, E. Fluorescence quenching in phenylalanine and model compounds. *Photochem. Photobiol.* **16**, 425–433 (1972).
33. Feitelson, J. On mechanism of fluorescence quenching. Tyrosine + similar compounds. *J. Phys. Chem.* **68**, 391–397 (1964).
34. Schafer, K., Goddinger, D. and Hocker, H. Photodegradation of tryptophan in wool. *J. Soc. Dye. Colour.* **113**, 350–355 (1997).
35. Arnaud, R., Perbet, G., Deflandre, A. and Lang, G. Electron-spin-resonance study of hair and melanin keratin mixtures – the effects of temperature and light. *Int. J. Cosmet. Sci.* **6**, 71–83 (1984).
36. Robbins, C.R. and Bahl, M.K. Analysis of hair by electron-spectroscopy for chemical-analysis. *J. Soc. Cosmet. Chem.* **35**, 379–390 (1984).
37. Bent, D.V. and Hayon, E. Excited-state chemistry of aromatic amino-acids and related peptides. 3. Tryptophan. *J. Am. Chem. Soc.* **97**, 2612–2619 (1975).
38. Pailthor, M.T. and Nicholls, C.H. Esr studies of low-temperature irradiation of keratin and its component amino-acids. *Photochem. Photobiol.* **15**, 465–477 (1972).
39. Frisch, M.J., Trucks, G.W., Schlegel, H.B. *et al. Gaussian 98 (Revision A.1)*. Gaussian, Inc., Pittsburg, PA (1998).
40. Lee, C.T., Yang, W.T. and Parr, R.G. Development of the colle-salvetti correlation-energy formula into a functional of the electron-density. *Phys. Rev. B. Condens. Matter* **37**, 785–789 (1988).
41. Becke, A.D. Density-functional thermochemistry. 3. The role of exact exchange. *J. Chem. Phys.* **98**, 5648–5652 (1993).
42. Pople, J.A., Headgordon, M. and Raghavachari, K. Quadratic configuration-interaction – a general technique for determining electron correlation energies. *J. Chem. Phys.* **87**, 5968–5975 (1987).

43. Morpurgo, S., Bossa, M. and Morpurgo, G.O. Ab initio study of intramolecular proton transfer reactions in cytosine. *Chem. Phys. Lett.* **280**, 233–238 (1997).
44. Sambrano, J.R., Andres, J., Beltran, A., Sensato, F. and Longo, E. Theoretical study of the structure and stability of NbxOy, and NbxOy+ (x = 1–3; y = 2–5, 7, 8) clusters. *Chem. Phys. Lett.* **287**, 620–626 (1998).
45. Sambrano, J.R., de Souza, A.R., Queralt, J.J., Olive, M. and Andres, J. Density functional study of the 5-methylcytosine tautomers. *Chem. Phys.* **264**, 333–340 (2001).
46. Queralt, J.J., Andres, J., Canle, M., Cobas, J.H., Santaballa, J.A. and Sambrano, J.R. A joint theoretical and kinetic investigation on the fragmentation of (N-halo)-2-amino cycloalkanecarboxylates. *Chem. Phys.* **280**, 1–14 (2002).
47. Somers, K.R.F. and Ceulemans, A. Absorption and fluorescence of 3-methylindole: a theoretical study, including H2O interactions. *J. Phys. Chem. A* **108**, 7577–7583 (2004).
48. Pilen, M.P., Santus, R. and Land, E.J. Photosensitizing properties of N-Formylkynurenine and related compounds. *Photochem. Photobiol.* **28**, 525–529 (1978).
49. Pileni, M.P., Giraud, M. and Santus, R. Kynurenic acid. 2. Photosensitizing properties. *Photochem. Photobiol.* **30**, 257–261 (1979).
50. Endo, K., Seya, K. and Hikino, H. Photo-oxidation of L-tyrosine, an efficient 1,4-chirality transfer-reaction. *J. Chem. Soc., Chem. Commun.* **14**, 934–935 (1988).