Journal of Photochemistry & Photobiology, B: Biology 164 (2016) 43–48

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Photoinduced formation of thiols in human hair

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ARTICLE INFO

Article history:
Received 7 July 2016
Accepted 12 September 2016
Available online 13 September 2016

Keywords:
Photodegradation
Photodamage
Hair keratins
Disulfide bonds
Sulfhydryl groups
Thiols
Raman microspectroscopy

ABSTRACT

Raman, scanning electron, and optical microscopy of hair and spectrophotometry of soluble hair proteins are used to study the effect of UV-vis radiation on white hair. The samples of a healthy subject are irradiated using a mercury lamp and compared with non-irradiated (control) hair. The cuticle damage with partial exfoliation is revealed with the aid of SEM and optical microscopy of semifine sections. Gel filtration chromatography shows that the molecular weight of soluble proteins ranges from 5 to 7 kDa. Absorption spectroscopy proves an increase in amount of thiols in a heavier fraction of the soluble proteins of irradiated samples under study. Raman data indicate a decrease in the amount of S—S and C—S bonds in cystines and an increase in the amount of S—H bonds due to irradiation. Such changes are more pronounced in peripheral regions of hair. Conformational changes of hair keratins presumably related to the cleavage of disulfide bonds, follow from variations in amide I and low-frequency Raman bands. An increase in the content of thiols in proteins revealed by both photometric data on soluble proteins and Raman microspectroscopy of hair cuts can be used to develop a protocol of the analysis of photoinduced hair modification.

1. Introduction

Hair cortex predominantly consists of fibrillar insoluble proteins (keratins) and low-molecular-weight proteins (21 families with relatively high content of cystein) [1,2,3].

Photodamage of hair keratins under UV irradiation involves photodegradation of aromatic amino acids (tryptophan, tyrosine, and phenylalanine) and cystine [4,5]. Hair weathering caused by sunlight depends on UV-induced destruction of cystine [6,7].

UV absorption leads to the transition of amino acids to excited states, and further reactions depend on pH of solution, temperature, polarity of neighboring side chains, and protein structure [4,8]. In particular, thiyl radical that is formed due to photon absorption by disulfide bond or interaction of disulfide bond with triplet tyrosines or tryptophans may abstract hydrogen atom from alanine or glycine and can be reduced to thiol [9,10]. UVB-induced formation of thiols was registered after in vitro irradiation of epidermal growth factor [11], plasminogen [12], apo-α-lactalbumin [13], and insulin [14]. Oxidation of thiyl radical leads to formation of sulfonic and sulfinic acids [4,6,15]. Photons can be absorbed by disulfide bonds of both low-molecular-weight soluble proteins and fibrillar keratins of cortex. The results of [3,16,17,18] show that hair damage by bleaching reagents as well as UV radiation causes an increase in the amount of soluble proteins extracted from hair shaft in aqueous medium.

Raman spectroscopy is known to be helpful in the study of protein structure. In particular, conformations of disulfide bridges and the secondary structure of protein molecules can be analyzed. The measurements in the low-frequency range make it possible to characterize conformational changes of a protein molecule as a whole [19]. Raman microspectroscopy can be used to study effect of irradiation on keratins using the analysis of Raman bands assigned to S—S, C—S, and S—H bonds. The presence of melanin, the granules of which absorb laser radiation and fluoresce, impedes the application of Raman spectroscopy [20,21]. For cuticle of unpigmented hair, the intensities of Raman bands assigned to S—S and C—S bonds are higher than the corresponding intensities for cortex [20]. Thus, we study effect of mercury-lamp irradiation on white hair of a healthy patient under laboratory conditions.

Optical microscopy and SEM are conventional methods used to study the structure of hair shaft, in particular, the surface roughness, scale thinning and fusion [22]. Gel-chromatography is a helpful tool in the study of soluble keratins in spite of their low content in hair extracts [23].

The purpose of this work is to prove cleavage of disulfide bonds of hair keratins resulting in the formation of thiols based on the experimental study of hair cuts and soluble proteins of hair.

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http://dx.doi.org/10.1016/j.jphotobiol.2016.09.021
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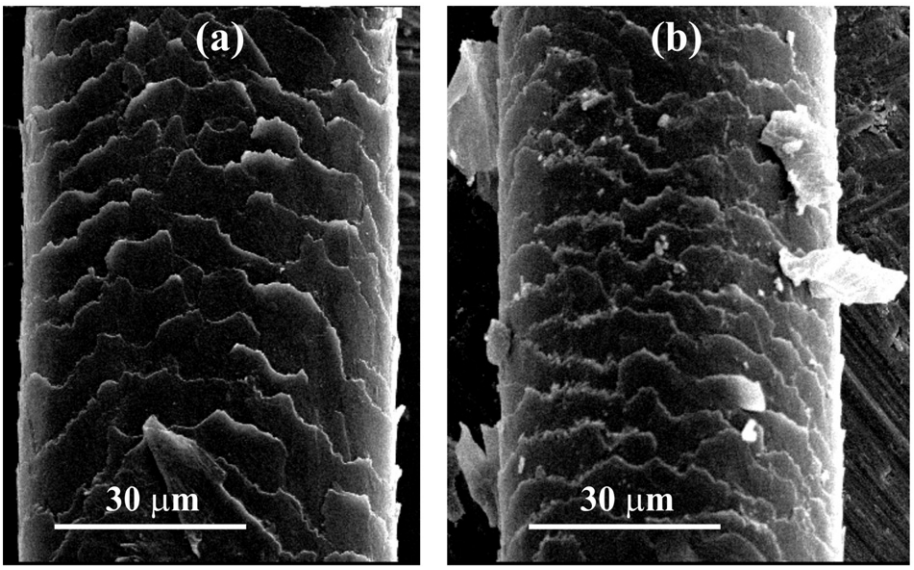

Fig. 1. SEM images of cuticle of (a) control and (b) irradiated hair shafts.

2. Materials and Methods

2.1. Samples

Unstained white (grey, unpigmented) hair from parietal region of a healthy patient were studied.

The following substances are used: Conducting Silver Paint (Ladd Research Industries); Romanovsky-Giemsa dye (Ecolab); araldite (Electron Microscopy Sciences); carbonate salts and Tris (Chimmed); PBS, DTNB (5,5′-dithio-bis(2-nitrobenzoic acid)), GSH (glutathione as a reduced form), bovine serum albumin (BSA), gel filtration molecular weight markers kit MW 12–200 kDa, soybean Bowman-Birk inhibitor, aprotinin from bovine lung, human insulin, and protamin from salmon (Sigma-Aldrich); and Total protein (Biuret) kit (Randox).

2.2. Irradiation

Hair samples are UV irradiated using a Medicor BLM-12 UV lamp. The spectrum of the lamp radiation is measured with the help of an Ocean Optics HR4000CG-UV-NIR spectrometer. The main emission bands of the lamp are picked at 254 (0.38), 366 (0.04), 406 (0.13), 437 (0.75), 547 (1), 578 (0.13), and 580 nm (0.13) (relative intensities of the bands are indicated in parentheses). The hair samples are placed at a distance of 15 cm from the lamp, where the intensity at a wavelength of 254 nm is about 30 μW/cm^2. (Note that the published results on the total sunlight intensity in the UVB region are 78 μW/cm^2 (280–315 nm) [24], 40 μW/cm^2 in December (below 313 nm) [25], and 175 μW/cm^2 in summer (below 313 nm) [25].) Radiation intensities are measured with the aid of a Coherent FieldMaster FM powermeter. Irradiation time is 6 h, so that the total energy density is 4.4 J/cm^2 and the energy density at a wavelength of 254 nm is 0.65 J/cm^2.

2.3. Scanning Electron and Optical Microscopy

A Hitachi S-570 scanning electron microscope is used for SEM measurements. The samples are placed on a sample stage and fixed using a Ladd Research Industries Conducting Silver Paint. The samples are covered with a 10-nm-thick layer of Pt-Pd alloy in an Eiko IB-3 Ion Coater.

For optical microscopy samples are processed using a conventional method for electron microscopy study with subsequent imbedding in araldite [26]. Semifine sections are obtained using a Reichert-Jung Ultracut ultramicrotome and stained with the aid of the Romanowsky-Giemsa procedure for histological analysis. A Nikon Eclipse E200 microscope with a DS-Fi1 camera head is employed.

2.4. Eluted and Soluble Hair Proteins

For the quantification of eluted proteins, 1–5 mg of hair fragments with a length of about 1 cm are incubated in 1 mL of 0.1 M carbonate buffer solution (pH 10.5) over 30 min in a shaker at room temperature. Then, the eluate is centrifuged at 900*g* over 20 min. Protein concentration is estimated using optical absorption at a wavelength of 240 nm

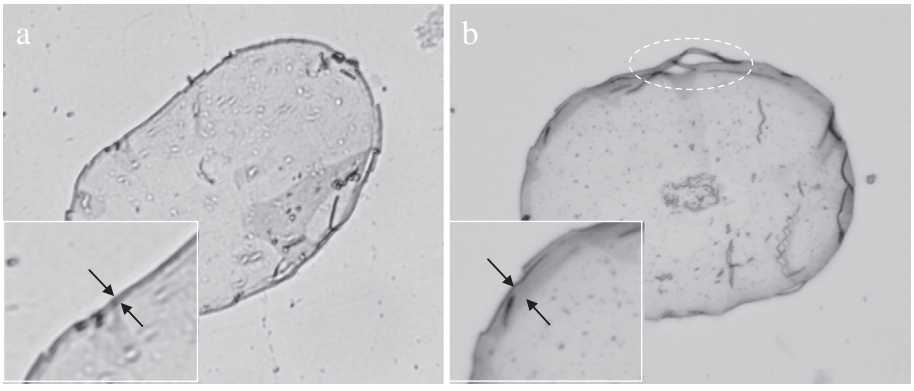

Fig. 2. Cuts of (a) control and (b) irradiated hair. The arrows show the estimated thickness of highly stained layers. The dashed line shows the fragment with exfoliated cuticle.