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Trace metal ions in hair from frequent hair dyers in China and the associated effects on photo-oxidative damage

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ABSTRACT

Human hairs are subject to oxidative modification when exposed to sunlight. In the present study, samples of human hair from Chinese volunteers that included frequent hair dyers and non-dyers were analyzed for metal ions such as iron, copper, magnesium, aluminum, zinc and lead. The generation of hydroxyl radicals during UVA (315–400 nm) photoageing was quantified and oxidative damages characterized by proteomic and SEM analysis. It was concluded that high levels of metal ions, particularly those derived from iron and copper, identified in the dyed hairs are associated with enhanced photoformation of hydroxyl radicals and resultant photooxidative damage of the hair. Reactive oxygen species, including hydroxyl radicals, generated via an electron transfer mechanism with hair photosensitizers react with hair proteins. Proteomic analysis of hair samples from frequent hair dyers, regardless of age and gender, showed an almost 1.6 fold increase in the protein oxidative modification levels compared to the undyed samples. As a result, a more pronounced physical damage including fragmentation and cross-linkage of cuticle scales was observed on the surface of dyed hair samples during the photoageing. This work is aimed at better understanding the role of metal ions in dyed hairs and their possible role in photosensitizing hair proteins. The results from this study are anticipated to contribute to the improved development of hair coloring cosmetics and hair care products.

1. Introduction

The use of hair dyes is widespread in Asian counties, particularly in China, Korea and Japan, as it allows consumers to either change hair color or cover gray in these aging societies. However, consumers who use artificial hair colors on a regular basis are often complaining of poor hair health, such as increased incidence of brittleness, splitting and color fading after long term exposure to sunlight.

The harmful effects of sun radiation on untreated human hair have been extensively reviewed [1–3]. The major mechanism is considered to be a photosensitized oxidation of the structural proteins (keratin) via the formation of activated reactive oxygen species (ROS) driven by the UVA radiation (315–400 nm) of the solar spectrum [2,3]. Incorporation of external catalysts, such as transition metal ions, can further increase the photosensitivity of the system and thereby significantly increase the rate of photodegradation. All transition metals which are capable of single-electron transfer in redox systems, particularly iron and copper, can catalyze the production of hydroxyl radicals by reaction with hydrogen peroxide via the Fenton chemistry [4]. Hydroxyl radicals are the strongest oxidizing species that can be produced in aqueous environment, and will rapidly react with virtually all organic molecules and/or initiate chain reactions, leading to significant photo-oxidation and photodegradation of proteinacous materials [5,6]. Previous studies with metal-doped wool, another keratin-based fiber, have associated iron and copper with enhanced production of hydroxyl radicals and increased phototendering of wool fibers [7].

It has largely been ignored that hair dye products also serve as an external source of metal ions which are mainly introduced as mordanting agents in the coloring system. A previous investigation in China has exhibited that the dyeing process can alter the levels of many transition metals and cause an increase in the concentration of iron, copper, magnesium, nickel and cadmium in the hair samples from female consumers [8]. A recent study by Grosvenor reported a correlation of enhanced oxidative modification in hair treated with increasing does of copper (II) ions, regardless of the pigmentation level of hair [9]. Incorporation of N,N′-ethylenediamine disuccinic acid, a copper-chelating agent, in the hair dyeing process has also been demonstrated to prevent the formation of oxygen radicals thus significantly reducing hair damage [10]. However, the relative levels of mordanting metal ions in hair

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and the associated effect on oxidative damage of hair during photoaging have not been thoroughly examined.

The purpose of this work is to investigate the relationship between accumulation of heavy metals, hydroxyl radical formation, protein oxidation and surface morphology of irradiated hair from frequent hair dyers. Advanced redox proteomic evaluation of molecular level modification has here been utilized in tandem with structural and elemental analysis techniques. The results are expected to contribute to the elucidation of photo-oxidative degradation mechanism of dyed hairs thus assisting the research and development of improved hair coloring systems and after-dye products.

2. Materials and Methods

2.1. Hair Mamples and Pre-treatment

Hair samples were collected during September to December of 2014 from 722 Chinese adult individuals, 20–80 years old and of both genders living at Tongzhou district, the south-eastern area of Beijing city. The hair donors were asked to complete a personal questionnaire to collect information about age, gender, ethnic origin, general health and hair-dyeing habits (frequent hair dyers or not) and their hairs were accordingly classified as dyed or undyed samples (Table 1). Each sample, dyed or undyed, was blended from hairs of donors of same gender and age group.

After sample collection, hair tresses were washed with 0.1% Triton X-100 in an ultrasonic bath for 15 min followed by three rinses with deionized distilled water and finally dried at 50 °C to a constant weight. Hair tresses from individual donors were snipped to approximately 5 mm in length to obtain a uniform size and thoroughly mixed before storing in polyethylene bags in the dark prior to use.

For each assay described, an identical experiment in the absence of hair was performed following identical experimental protocol. Measurements for each sample were carried out in triplicate on both irradiated samples and the dark controls.

2.2. Trace Metal Analysis by ICP–MS

0.5–2 g of each hair sample in triplicates was accurately weighed and put in a conical flask with the addition of 10 mL 69% HNO_3. After standing overnight, the acid was removed by evaporation on a hot plate at 120 °C to near dryness. 1 mL 70% $HClO_4$ was subsequently added and the temperature raised to 180 °C for 2 h. After cooling, the resulting residue was diluted with water to a final volume of 50 mL. Blanks were prepared following the same procedure, containing all reagents without hair sample.

The quantitative determination of metal elements in the hair samples were performed using an Agilent 7500ce inductively coupled plasma-mass spectrometer (ICP-MS, Agilent Technologies, USA). The instrument parameters are listed in Table 2.

Table 1
Human hair samples sourced from 722 Chinese individuals in Beijing*.

Undyed hairs				Dyed hairs			
Sample	Gender	Age	No. of donors	Sample	Gender	Age	No. of donors
M1	M	20–40	178	RM1	M	20–40	9
M2	M	40–60	108	RM2	M	40–60	91
M3	M	60–80	37	RM3	M	60–80	16
		Total no.	323			Total no.	116
F1	F	20–40	25	RF1	F	20–40	28
F2	F	40–60	35	RF2	F	40–60	126
F3	F	60–80	44	RF3	F	60–80	25
		Total no.	104			Total no.	179

* M = male, F = female.

Table 2
Conditions used for ICP-MS analysis.

ICP-MS parameter	Value
RF power	1500 W
RF matching	1.67 V
S/C temperature	2 °C
Sample depth	8.1 mm
Carrier gas flow rate	0.85 L/min
Make up gas flow rate	0.21 L/min
Nebulizer pump flow rate	0.10 rps

2.3. UVA-derived Production of Hydroxyl Radicals

Photogeneration of hydroxyl radicals in irradiated hair samples was determined using the method previously described by Millington with modifications [6]. In the analysis, non-fluorescent terephthalic acid dianion (TA) was used that reacts with hydroxyl radicals to form a fluorescent molecule, 2-hydroxyterephthalate (HTA, $\lambda_{ex} = 315$ nm, $\lambda_{em} = 425$ nm). UVA irradiation of hair samples was performed by immersing 150 mg hair samples in 3 mL freshly prepared 2 mM TA solution in a sealed 1 cm × 1 cm quartz cell which was then placed at a distance of 10 cm to two 15 W blacklight UVA fluorescence lamps (Spectronics XX-15 A, USA). The UV radiation ranged from 315 to 400 nm with a maximum output at 365 nm and a total flux in the range of 2.5–3.5 $mW \cdot cm^{-2}$. After irradiation, the fluorescence of HTA was immediately recorded by a Cary-Eclipse fluorescence spectrophotometer (Varian, Cary, USA). The cells were checked before fluorescence measurement to make sure all the hair samples subsided to the bottom without any suspended hair blocking the light path. Triplicate analysis for both irradiated and unirradiated hair samples were performed. Irradiated TA solutions were used as controls.

All chemicals used were analytical reagent grade and were obtained from Sigma-Aldrich. Solutions of TA and HTA were prepared in 50 mM phosphate buffer (KH_2PO_4–KOH, pH 7.6).

2.4. Proteomic Analysis by LC–MS

2.4.1. Sample Preparation

Sub-samples of each hair sample were extracted using an extraction buffer (7 M urea, 2 M thiourea and 50 mM dithiothreitol (DTT), pH 7.5) at room temperature for 18 h using vigorous shaking. The extraction buffer components were then removed from the samples using a methanol chloroform precipitation step. For trypsin digestion, the precipitated proteins along with the extraction buffer treated hair were reduced using 50 mM TCEP in 100 mM ammonium bicarbonate buffer (pH 8.5) for 1 h at room temperature followed by alkylation with 150 mM iodoacetamide in 100 mM ammonium bicarbonate buffer (pH 8.5) for 30 min at room temperature in the absence of light. Each sample was extracted and analyzed in triplicate. The reduced and alkylated proteins were digested for 18 h at 37 °C with 10 μL of sequencing grade trypsin (Promega) at a concentration of 1 mg/mL in 100 mM ammonium bicarbonate buffer. The peptides thus generated were used for mass spectrometry analysis.

Table 3
List of amino acid targets used for oxidative modification searches.

Modification	Target amino acids	Position	Chemical modification
Single oxidation	CMFWY	Any	O(1)
Dioxidation	CFWY	Any	O(2)
Trioxidation	C	Any	O(3)
Nitration	WY	Any	H(−1)N(1)O(2)
Kynurenine	W	Any	C(−1) O(1)
Hydroxykynurenine	W	Any	C(−1)O(2)