Received: 7 July 2021 | Accepted: 14 October 2021

DOI: 10.1111/ics.12743

International Journal of Cosmetic Science
SOCIÉTÉ FRANÇAISE DE COSMÉTOLOGIE

RESEARCH COMMUNICATION

Impact of protein carbonylation on the chemical characteristics of the hair surface

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Abstract

Objective: The purpose of this study was to clarify the impact of protein carbonylation on the chemical characteristics of the hair surface focusing on hydrophobicity.

Methods: First, we examined the validity of methods to evaluate hydrophobicity, one that utilizes the fluorescence of 1-anilinonaphtalene-8-sulfonic acid (1,8-ANS) compared with the contact angles against H_2O, of the hair surface chemically modified by alkaline hydrolysis or treated with stearyl ammonium chloride. We measured hairs bleached with H_2O_2 or treated with acrolein for fluorescence originating from 1,8-ANS, for the contact angle and for changes of functional groups, aldehydes (the degree of carbonylation), NH_2, COOH and SH, using fluorescence labelling methods.

Results: The fluorescence intensity of 1,8-ANS of the hair surface modified chemically correlated well with the contact angles against H_2O. The results indicated that 1,8-ANS is suitable for evaluating the hydrophobicity of the hair surface. The hydrophobicity of hairs bleached with H_2O_2 or carbonylated with acrolein was decreased. In addition, changes of functional groups in hairs carbonylated with acrolein increased as did those of hairs bleached with H_2O_2.

Conclusion: The results suggest that the carbonylation of proteins at the hair surface with aldehydes decreases hydrophobicity and promotes further damage as does bleaching.

KEYWORDS

acrolein, carbonylation, H_2O_2, hair, hydrophobicity

Résumé

Objectif: l'objectif de cette étude était de clarifier l'impact de la carbonylation des protéines sur les caractéristiques chimiques de la surface des cheveux en se concentrant sur l'hydrophobicité.

Méthodes: nous avons d'abord examiné la validité de la méthode d'évaluation de l'hydrophobicité, une méthode qui utilise la fluorescence de l'acide 1-anilinonaph talène-8-sulfonique (1,8-ANS) par rapport aux angles de contact avec l'H2O, de la surface des cheveux chimiquement modifiés par hydrolyse alcaline ou traités par

chlorure d'ammonium stéarylique. Nous avons mesuré la fluorescence provenant du 1,8-ANS, l'angle de contact et les modifications des groupes fonctionnels, aldéhydes (le degré de carbonylation), NH2, COOH et SH des cheveux décolorés à l'H2O2 ou traités par acroléine, à l'aide de méthodes de marquage par fluorescence.
Résultats: l'intensité de la fluorescence du 1,8-ANS de la surface des cheveux modifiés chimiquement était bien corrélée aux angles de contact avec l'H2O. Les résultats ont indiqué que le 1,8-ANS était adapté à l'évaluation de l'hydrophobicité de la surface des cheveux. L'hydrophobicité des cheveux décolorés à l'H2O2 ou carbonylés à l'acroléine a diminué. De plus, les modifications des groupes fonctionnels des cheveux carbonylés par l'acroléine ont augmenté, tout comme celles des cheveux décolorés à l'H2O2.
Conclusion: les résultats suggèrent que la carbonylation des protéines à la surface des cheveux par des aldéhydes diminue l'hydrophobicité et favorise d'autres dommages, tout comme la décoloration.

INTRODUCTION

The hair surface has substantial hydrophobic characteristics due to the lipid layer, also called the f-layer, at the surface of cuticles, which is composed of covalently bound 18-methyleicosanoic acid (18-MEA) and by the presence of free fatty acids [1]. The main function of the epicuticle is to create a water-repellent surface that makes hair waterproof and prevents the tangling of wet hairs due to the reduced attraction to water.

Hairs are always exposed to external stimuli such as daily weather, shampoos and blowing hot air for drying. Furthermore, hairs also suffer from chemical modifications by treatment with permanent styling and colouring in order to obtain individual satisfaction according to contemporary fashion trends. Among those stimuli, the bleaching of hair with H_2O_2 at an alkaline pH for colouring decreases the hydrophobicity of hairs and results in damaged hairs through the structural decomposition of the cuticle and the leakage of proteins, showing a lower contact angle against H_2O [2–4]. Among external stimuli, daily solar ultraviolet light (UV) is well-known as a generator of reactive oxygen species (ROS) [5] and also damages hairs [6,7]. Considering their anatomical structure, hairs contain abundant lipids in sebum, because skin pores are associated with sebaceous glands. Thus, hairs always face the threat of lipid oxidation by UV-generated ROS. In general, aldehydes that are synthesized during lipid peroxidation due to reactions with unsaturated lipids and ROS, initiate protein carbonylation by reacting with amino groups in lysine and arginine residues of proteins [8]. In fact, it has been reported that sunlight causes the chemical modification of proteins in hairs such as the formation of carbonyl groups [7]. In other words, hairs might be in a circumstance where they are continually exposed to aldehydes.

On the other hand, protein carbonylation in the stratum corneum of skin has been reported to reduce the moisture function such as a negative correlation with skin surface water content, a positive correlation with trans-epidermal water loss, and by reducing bound water [9]. Thus, it has been assumed that the oxidative modification of proteins in corneocytes such as carbonylation decreases hydration in the skin. However, knowledge of the chemical properties of the hair surface carbonylated by aldehydes is still poor.

1-Anilinonaphtalene-8-sulfonic acid (1,8-ANS) has been used as a florescent probe to measure the hydrophobic characteristics of lipid membranes [10]. Thus, we expected that 1,8-ANS could be used to evaluate hydrophobicity at the hair surface. In this study, we first validated whether the fluorescence that originates from 1,8-ANS is appropriate to evaluate the hydrophobicity of the hair surface by comparing the contact angle against H_2O. Second, we measured the chemical characteristics of hairs carbonylated with aldehyde by comparing those to hairs bleached with H_2O_2. In this study, we chose acrolein, which is an α,β-unsaturated aldehyde, since acrolein is produced by UV-induced lipid peroxidation [11] and acrolein-derived carbonylated proteins are found in the stratum corneum at a higher frequency [12]. Thus, we thought that acrolein is one of the aldehydes that produce carbonylated proteins at the hair surface.

MATERIALS AND METHODS

Reagents

Strands of Asian black hairs (hereafter called tresses) were made with hairs purchased from Beaulax (Saitama, Japan). Acrolein, stearyl amine, 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl), maleimide and 1-(3-dimethylaminopropyl)-3-et

hylcarbodiimide hydrochloride (EDC) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Fluorescein-5-thiosemicarbazide (FTSC), N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide (DACM), dansyl cadaverine (DC) and 1,8-ANS were purchased from Sigma-Aldrich (Darmstadt, Germany). Tris(hydroxymethyl)aminomethane (Tris), 2-(N-morpholino) ethane sulfonic acid (MES), acetic acid, sodium hydroxide (NaOH), hydrogen chloride (HCl), 30% H_2O_2, 28% ammonia solution and phosphoric acid were purchased from Nacalai Tesque (Kyoto, Japan). Stearyl *tri*-methylammonium chloride (LIPOTHOQUAD T-800) was a kind gift from Kimura Sangyo Co., Ltd (Tokyo, Japan). *penta*-Sodium diethylene *tri*-amin *penta*-acetic acid (CLEWAT DP-80) was a gift from Sasaki Chemicals Inc. (Tokyo, Japan).

Chemical modification of hair hydrophobicity

Treatment with NaOH

In order to remove hydrophobic moieties on the surface of hair, a hair tress was incubated with NaOH at concentrations of 10 mM and 100 mM for 2 h at 60°C. The hair tress was then washed with deionized H_2O and was dried to be used for subsequent fluorescence labelling.

Treatment with stearyl ammonium chloride

A hair tress was incubated with an aqueous solution containing stearyl ammonium chloride at concentrations of 50, 500 μM and 5 mM for 1 h at room temperature. After washing with deionized H_2O, the hair tress was used for subsequent examinations. Stearyl ammonium chloride was prepared by neutralizing stearyl amine with HCl.

Assessment of hydrophobicity

Fluorescence of 1,8-ANS

A hair tress was incubated with an aqueous solution containing 100 μM 1,8-ANS for 10 min at room temperature in the dark after hydration with deionized H_2O for 10 min at room temperature. Fluorescence was measured using a Floid Cell Imaging Station (Life Technologies Corp., NY, USA), and fluorescence intensities (FI) were quantified using specialized corneocytometry software (CIEL, Tokyo, Japan).

Measurement of the contact angle

In order to assess the hydrophobicity of the hair surface, the contact angle of deionized H_2O dropped on hair was measured. Two microliter of deionized H_2O was placed on a flat plate on which 10 hairs were arranged. A photo of the H_2O droplet was taken from the horizontal side (Figure 1). The contact angle of the H_2O droplet on the hairs was measured using the photo.

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Treatment with acrolein

A hair tress was immersed in an aqueous solution containing acrolein at concentrations of 1, 10 and 100 mM, and was incubated for 24 h at 37°C. The hair tress was then washed three times with deionized water and was dried and then used for subsequent fluorescence labelling.

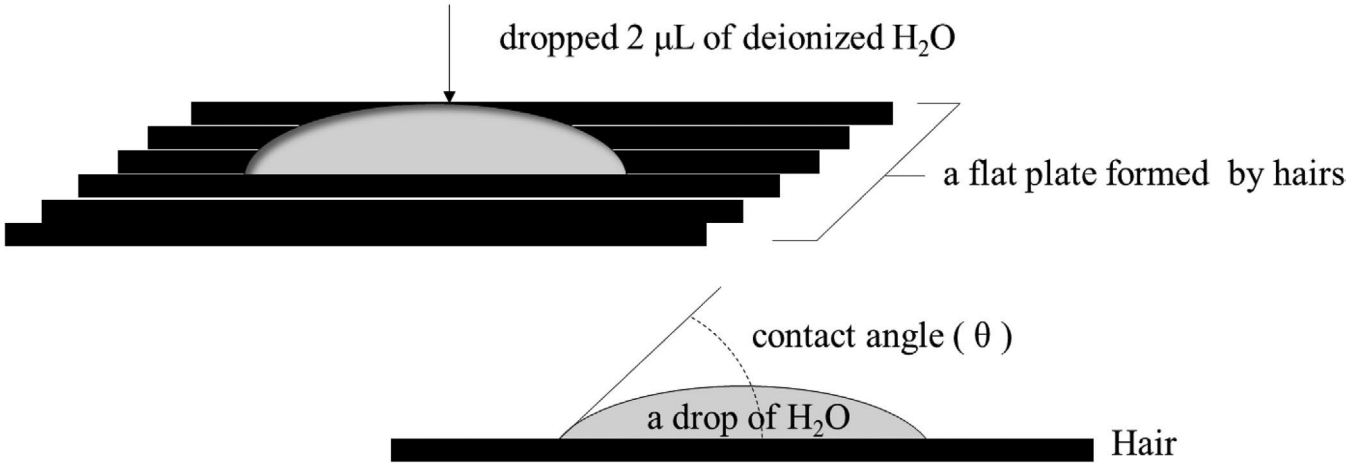

FIGURE 1 Contact angle of deionized H_2O_2 against hairs on a flat plate

Hair bleaching with H_2O_2

A hair tress was bleached with H_2O_2 and was then treated with the same amount of a mixture of formulations 1 and 2, which are shown in Table 1, for 20 min at 37°C. To prepare the bleached hair tress, the process was repeated five times.

Fluorescence labelling of functional groups in proteins of hair

Aldehyde groups were visualized by fluorescence labelling using FTSC. A hair tress was hydrated by immersion in 0.1 M MES-Na buffered solution (pH 5.5) for 10 min at room temperature, and was then immersed in 0.1 M MES-Na buffered solution (pH 5.5) containing 20 μM FTSC for 1 h at room temperature [13]. Amino (NH_2) groups were visualized by fluorescence labelling with NBD-Cl after blocking sulfhydryl (SH) groups with maleimide. A hair tress was hydrated by immersion in Tris-HCl buffered solution (pH 6.8) for 10 min at room temperature, and then was incubated in Tris-HCl buffered solution (pH 6.8) containing 2 M maleimide overnight at 37°C to block SH groups. After washing with deionized H_2O, the hair tress was incubated with 0.1 M $NaHCO_3$ aqueous solution containing 25 μM NBD-Cl for 2 h at room temperature. Carboxylic (COOH) groups were visualized by fluorescence labelling with dansyl cadaverine. A hair tress was hydrated by immersion in 0.1 M MES-Na buffered solution (pH 5.5) for 10 min at room temperature, and then by immersion in 0.1 M MES-Na buffered solution (pH 5.5) containing 100 μM dansyl cadaverine in the presence of 1 mM EDC for 2 h at room temperature [14]. SH groups were visualized by fluorescence labelling with DACM. A hair tress was hydrated by immersion in 0.1 M TAS buffered solution (pH 6.8) for 10 min at room temperature, and then by immersion in 0.1 M TAS buffered solution (pH 6.8) containing 10 μM DACM for 3 min at room temperature [15]. Fluorescence images were obtained using a Floid Cell Imaging Station (Life Technologies Corp., NY, USA). Fluorescence intensities in fluorescence images were quantified using specialized corneocytometry software (CIEL, Tokyo, Japan).

TABLE 1 Formulations for hair bleaching

	% w/w
Formulation 1	
Stearyl *tri*-methylammonium chloride	2.00
28% ammonium aqueous solution	10.00
Penta-sodium diethylene *tri*-amin *penta*-acetic acid	0.10
Deionoized H_2O	87.90
	100.00
Formulation 2	
30% hydrogen peroxide aqueous solution	20.00
Penta-sodium diethylene *tri*-amin *penta*-acetic acid	0.10
85% phosphoric acid aqueous solution	0.08
Deionoized H_2O	79.82
	100.00

Statistical analysis

All data are expressed as means ± SD. Comparisons between two groups were performed using Student's *t*-test and were performed between multiple groups using the Tukey-Kramer test. A p-value < 0.05 is considered statistically significant.

RESULTS

Validation of the hydrophobicity assessment of hair by the fluorescence intensity of 1,8-ANS

As a fundamental structure of hair, the 18-MEA covalently bound at the surface of the hair as a thioester [1], and free fatty acids are responsible for the hydrophobicity of hair. To modify the hydrophobicity of hair, some moieties contributing to the hydrophobic characteristic, such as 18-MEA and free fatty acids, was removed by treatment with NaOH. In fact, the fluorescence intensity (FI) of 1,8-ANS treated hairs were significantly decreased in a concentration-dependent manner by treatment with NaOH (Figure 2). On the other hand, the contact angle of a H_2O droplet on NaOH-treated hairs decreased significantly according to the treatment conditions. In contrast, treatment of hair with stearyl ammonium chloride significantly increased the FI of 1,8-ANS and significantly increased the contact angle according to the concentration of stearyl ammonium chloride (Figure 2).

Impact of protein carbonylation on the chemical characteristics of hair

Bleaching with H_2O_2 caused the carbonylation of proteins in hair by significantly increasing aldehyde groups, NH_2 groups, COOH groups and SH groups, all of which are hydrophilic (Figure 3). Hairs treated with acrolein also gave a similar behaviour by changing the functional groups in proteins of hairs (Figure 4), which are expected to influence the hydrophobicity of hairs. In fact, the FI of

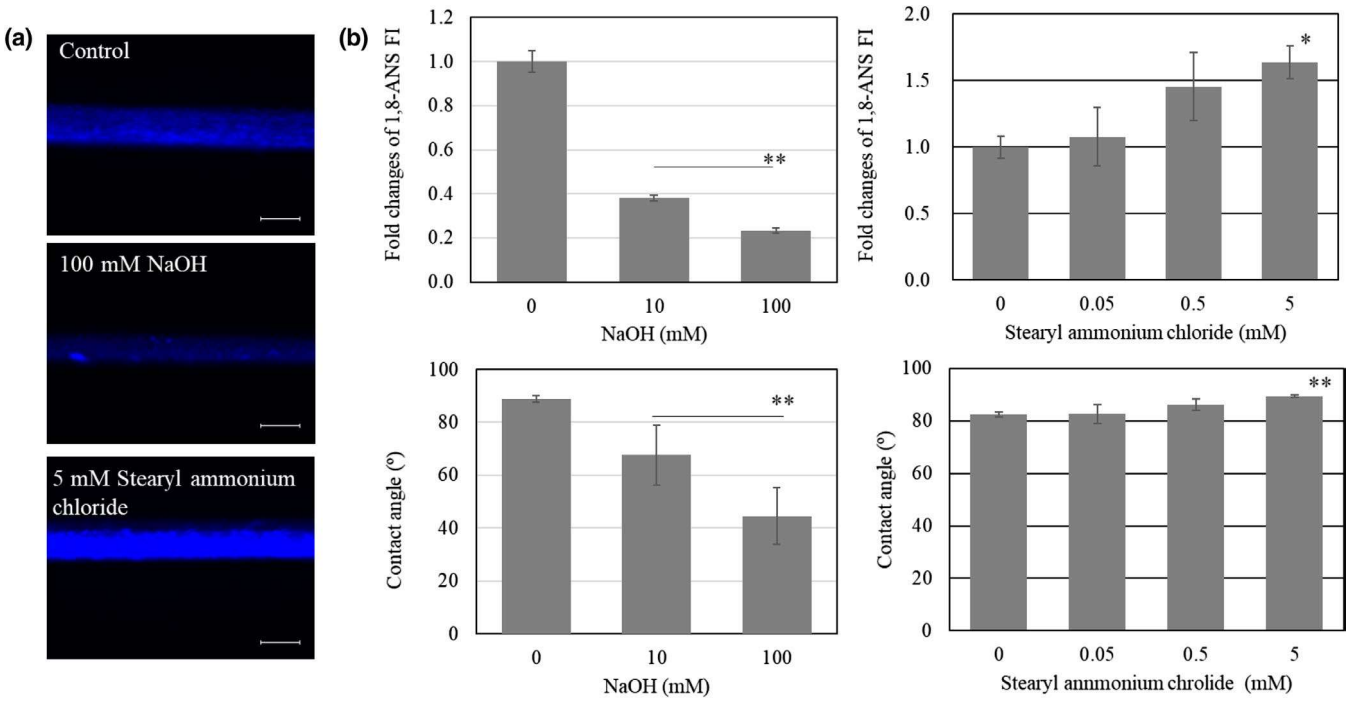

FIGURE 2 Fluorescence intensity of 1,8-ANS and contact angle in chemically modified hairs. Hairs were treated with NaOH for 2 h at 60°C or with stearyl ammonium chloride for 1 h at room temperature. (a) Representative fluorescence images of hairs treated with 100 mM NaOH or with 5 mM stearyl ammonium chloride. Scale bars: 100 μm. (b) Digitalized data of the FI of 1,8-ANS and the contact angle of hairs treated with different concentrations of NaOH or stearyl ammonium chloride. Columns show means ± SD. Experiments were carried out in quadruplicate. Data were analysed using the Tukey-Kramer test. Significance $^{*}p < 0.05$, $^{**}p < 0.01$ versus the control (0) [Colour figure can be viewed at wileyonlinelibrary.com]

1,8-ANS was significantly reduced depending on the concentration of acrolein and by bleaching with H_2O_2, which is associated with significant reductions of the contact angle against H_2O. Gathering these results, we conclude that protein carbonylation caused by treatment with acrolein or with H_2O_2 reduced the hydrophobicity of hairs by increasing hydrophilic functional groups.

DISCUSSION

Hairs are frequently chemically modified to satisfy the demands of fashion trends, and can also be unconsciously damaged due to exposure to environmental stimuli. The current study was carried out to clarify the impact of protein carbonylation, which is a kind of oxidative modification, on the chemical characteristics of the hair surface. Hairs have hydrophobic characteristics at their surface due to 18-MEA bound to sulfhydryl groups [1]. The hydrophobic characteristic of hair is probably responsible for giving lustre to hairs optically by facilitating the spread of sebum and for preventing protein leakage from inside of hairs due to interfering with the penetration of H_2O. Thus, maintaining the hydrophobicity of hairs is a crucial issue to maintain the health of hairs, because losing proteins leads to a fragile structure, and results in decreased resistance against mechanical stress. Therefore, in this study, we evaluated the hydrophobicity of the hair surface as a chemical characteristic.

First, the possibility of measuring fluorescence originating from 1,8-ANS to evaluate the hydrophobicity of hairs was examined, since 1,8-ANS has been used as a florescent probe to measure hydrophobicity [10]. It is thought that treatment with NaOH removes the covalent binding of 18-MEA to the hair surface and removes free fatty acids, which would result in a lower hydrophobicity. The results showed that hairs treated with NaOH had a lower contact angle against H_2O (Figure 2). In general, alkyl quaternary ammonium salts are used to condition hairs by adsorption through ionic interactions. Thus, treatment of hairs with stearyl ammonium chloride was expected to increase hydrophobicity. In fact, hairs treated with stearyl ammonium chloride showed a higher contact angle against H_2O indicating an enhanced hydrophobicity (Figure 2). In addition, the FI of 1,8-ANS on the hair surface treated with NaOH or with stearyl ammonium chloride gave behaviours corresponding to changes of the contact angle (Figure 2), because the correlation coefficients between the FI of 1,8-ANS and the contact angle of hairs treated with NaOH or with stearyl ammonium

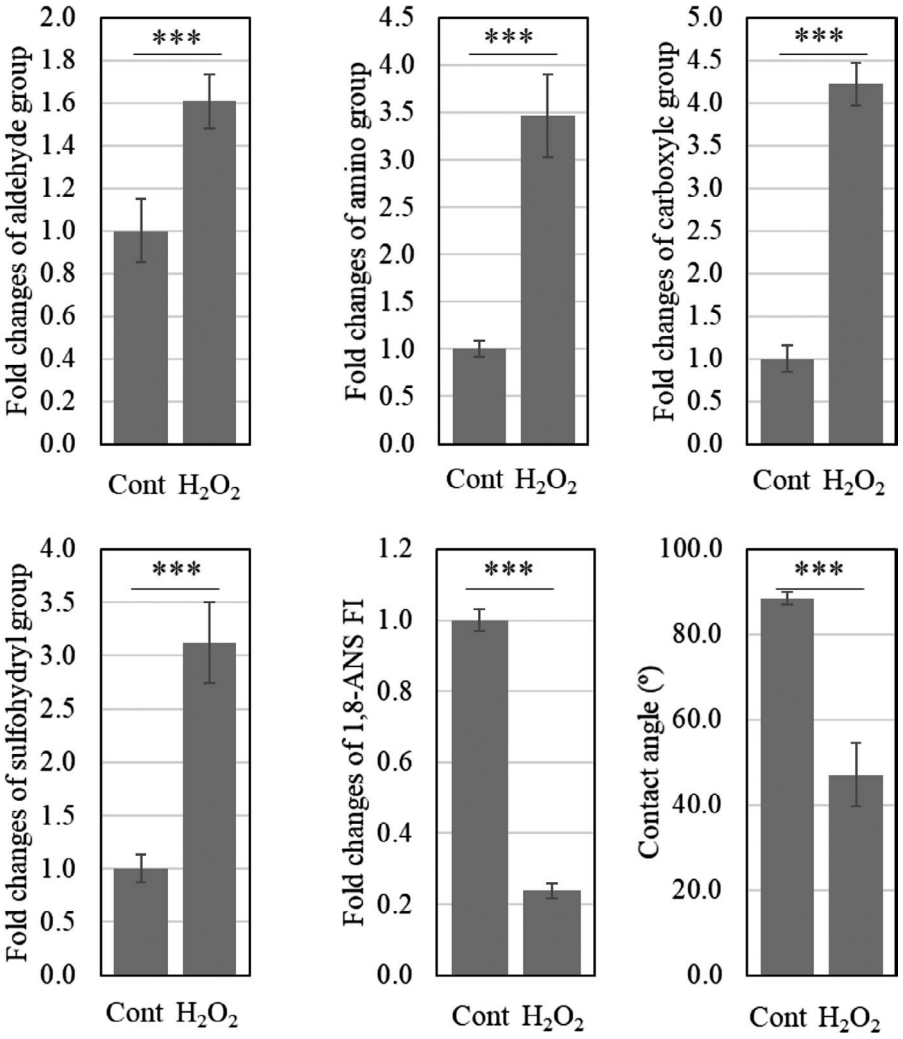

FIGURE 3 Changes of functional groups, fluorescence intensity of 1,8-ANS and contact angle in hairs bleached with H_2O_2. Hairs were bleached by five treatments with H_2O_2 for 20 min at 37°C, and were then fluorescence labelled with each corresponding reagent after washing. Hairs untreated with H_2O_2 were used as a control. Changes of functional groups and the FI of 1,8-ANS were digitalized by image analysis of the fluorescence images. Columns show means ± SD. Experiments were carried out in quadruplicate. Data were analysed using Student's *t*-test. Significance ***$p < 0.001$ versus the untreated control (Cont)

chloride were 0.934 and 0.986 respectively. The sum of these results indicated that the FI of 1,8-ANS is a useful tool to measure the hydrophobicity of the hair surface.

Before discussing the impact of aldehyde on hair characteristics, we summarize the changes of chemical characteristics of hairs bleached with H_2O_2. Hair damage caused by bleaching with H_2O_2 has been well studied from morphological and physical viewpoints. H_2O_2 damages hairs such as making their cuticles more porous, reducing their smoothness and decreasing their lustre [3,4]. Furthermore, bleaching hairs with H_2O_2 reduces the hydrophobicity of the hair surface [2]. In this study, we found that treatment of hairs with H_2O_2 increased protein carbonylation, and reduced the contact angle and FI of 1,8-ANS (Figure 3). Those results indicated that hairs bleached with H_2O_2 have a lower hydrophobicity and also verified the method of using 1,8-ANS to evaluate the hydrophobicity of hair.

Similarly, treatment with acrolein also decreased the hydrophobicity of hair indicating an increase of protein carbonylation and reductions of both the contact angle and the FI of 1,8-ANS (correlation coefficient: 0.951) (Figure 4). Those results suggested that protein carbonylation with aldehydes yielded during lipid peroxidation by exposure to environmental stimuli such as UV decrease the hydrophobicity of the hair surface.

In order to clarify the mechanism involved in decreasing the hydrophobicity of hair, changes of the functional groups in hair proteins were examined. Hairs bleached with H_2O_2 or carbonylated with aldehyde showed similar increases of NH_2, COOH and SH groups (Figures 3 and 4). Because those functional groups are hydrophilic, we considered that the increases of hydrophilic functional groups are responsible for decreasing the hydrophobicity of the hair surface. Considering the changes of functional groups by treatment with H_2O_2 or with aldehyde, the increase of COOH groups might be understood to be derived from further oxidation of aldehydes in carbonylated proteins and the hydrolysis of ester bonds. It has been reported that NH_2 groups yield aldehyde in the presence of metals by reaction with H_2O_2 [16]. On the other hand, SH groups generally generate thioacetal by reacting with

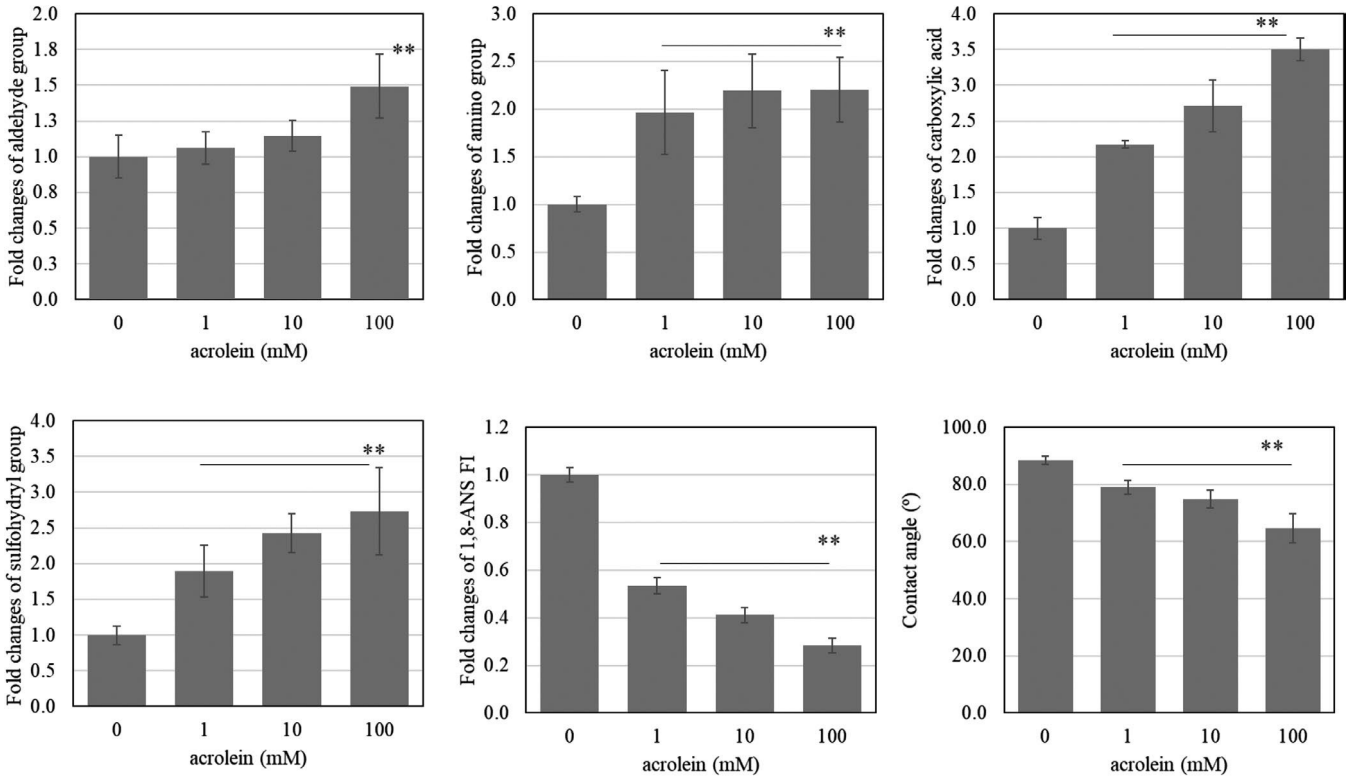

FIGURE 4 Changes of functional groups, fluorescence intensity of 1,8-ANS and contact angle in hairs carbonylated with acrolein. Hairs were immersed in an aqueous solution containing different concentrations of acrolein for 24 h at 37°C, and were then fluorescence labelled with each corresponding reagent after washing. Changes of functional groups and the FI of 1,8-ANS were digitalized by image analysis of the fluorescence images. Columns show means ± SD. Experiments were carried out in quadruplicate. Data were analysed using the Tukey-Kramer test. Significance $^{**}p < 0.01$ versus the control (0)

aldehydes. Gathering these facts, NH_2 and SH groups should decrease in hairs treated with H_2O_2 or aldehyde. The reasons for those increases might be as follows: The increase of NH_2 groups suggested that the hydrolysis of amide bonds occurs in hair oxidized with H_2O_2 or treated with acrolein, while the increase of SH groups also suggested that disulphide bonds might be cleaved. However, the reaction mechanisms involved are still unclear. Thus, we will continue this study and will report the results elsewhere.

CONCLUSION

In this study, the impact of protein carbonylation on the hydrophobicity of hairs was examined, and the usefulness of measuring the FI of 1,8-ANS to evaluate the hydrophobicity of hairs was verified. Furthermore, the results indicated the possibility that aldehydes promote further hair damage by lowering the hydrophobicity of hairs as well as bleaching. In the future, we will continue this study to clarify the impact of aldehyde-induced hair damage on the physical characteristics of hairs.

ACKNOWLEDGEMENTS

We thank Mr. Masahiko Ikeo, CEO of CIEL Co., Ltd. for allowing us to publish this study.

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How to cite this article: Masaki H, Sinomiya D, Okano Y, Yoshida M, Iwabuchi T. Impact of protein carbonylation on the chemical characteristics of the hair surface. Int. J. Cosmet. Sci. 43, 764–771 (2021). https://doi.org/10.1111/ics.12743