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Human hair keratin responds to oxidative stress via reactive sulfur and supersulfides

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ABSTRACT

Keratin is a central component of human hair proteins, which explicitly possesses many cysteine residues (Cys-SH). For a long time, these Cys-SH residues were believed to contribute to human hair strength by forming intra- and inter-molecular disulfide bond crosslinks. However, we detected that many polysulfide bonds (R-$SS_{(n)}$H or R-$SS_{(n)}$S-R') exist in keratin. Polysulfide is one of the reactive sulfur and supersulfides, similar to cysteine persulfide (Cys-SSH), that regulates oxidative stress and redox signaling. In the present study, we elucidated the distribution of polysulfide in human hair and the reaction of polysulfide to various oxidative stress, such as heat shock and ultraviolet radiation. The decrease of the polysulfides in hair leads to the loss of antioxidant activity. Additionally, we demonstrated the effect of sulfur supplementation on human hair strength and hair cuticle structure. All types of oxidative stresses decreased the polysulfide in human hair, and hair polysulfide positively correlated with human hair strength. Intriguingly, sulfur supplementation improved human hair strength and the structure of hair cuticles. In conclusion, polysulfide in human hair keratin contributes to hair strength and antioxidant activity against oxidative stresses such as ultraviolet radiation and maintains hair homeostasis.

Abbreviations

RSS	reactive sulfur and supersulfides
Cys-SSH	cysteine persulfide
CSE	cystathionine-γ-lyase
CBE	cystathionine-β-synthase
CARS	cysteine tRNA synthetase
EMSP	elimination method of sulfide from polysulfide
SSP4	sulfane sulfur probe-4
DMSO	dimethyl sulfoxide
DTNB	5,5-dithiobis-2-nitrobenzoic acid
CTAB	hexadecyltrimethylammonium bromide
DPDA	N,N-dimethyl-p-phenylenediamine
OCT	optimal cutting temperature
DPPH	2,2-Diphenyl-1-picrylhydrazyl
SEM	scanning electron microscopy.

Introduction

Human hair consists of a three-layer structure; the innermost layer of the hair is the medulla, the middle layer is the cortex, and the cuticle is the outermost layer. The cortex comprises various fibers contributing to the human hair's thickness, elasticity, and flexibility [1]. Keratin is a main fiber component of human hair protein. Approximately 90 % of keratin is mainly contained in the cortex and cuticle [2]. Most hair keratins contain cysteine-rich proteins of approximately 15 % cysteine (Cys-SH) and cysteine forming disulfide bonds (Cys-SS-Cys). These disulfide bonds contribute to the toughness of the human hair through the formation of cortical fibers and the shape of the human hair. Permanent waving agents cause the structural transformation of hair via shuffling

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disulfide bonds by oxidizing/reducing disulfide bonds in the human hair [3]. It is generally believed that oxidative stresses such as ultraviolet (UV) cleave the disulfide bonds in keratin, and this damage leads to hair breakage and split ends [4]. Therefore, understanding the role of disulfide bonds in keratin and exploring protecting substances for maintaining the toughness of human hair have been studied for a long time [5,6].

Recently, reactive sulfur and supersulfides (RSS), such as cysteine persulfide (Cys-SSH) and polysulfide (R-SS_nH or R-SS_nS-R'), were found to be present as intra- and extracellular components in mammals [7]. RSS includes low molecular weight compounds such as amino acids, glutathione, cysteine, persulfide, and polysulfide compounds in proteins. Polysulfide compounds are highly potent antioxidants and protect against reactive oxygen species [7]. Thus, polysulfide compounds are involved in the oxidative defense mechanism. Cysteine persulfide is synthesized by enzymes such as cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS), and cysteine tRNA synthetase (CARS) [7,8]. These enzymes actively and constitutively generate polysulfide, which is essential in the homeostasis maintenance mechanism in vivo. In particular, CARS synthesizes Cys-SSH using cysteine as a substrate and incorporates Cys-SSH into the protein during translation to produce protein persulfide [8].

Regarding polysulfide measurement methods reported so far, most studies measure the 'reduced' polysulfide (R-SSnH), such as the tag-switch method [9,10], the modified biotin switch method [11], and the ProPerDP method [12]. On the other hand, the 'oxidized' polysulfide (R-SS_nS-R') has been semiquantitatively detected using sulfane sulfur probe-4 (SSP4) [13]. We previously demonstrated that the sulfane sulfur atoms in 'oxidized' polysulfide were released as sulfide ions when treated with ascorbic acid in an alkaline solution [14]. The eliminated sulfide ions can be measured quantitatively using the conventional methylene blue method. Thus, the method was named "Elimination Method of Sulfide from Polysulfide (EMSP)." Using this simple method such as EMSP, polysulfide could be detected in human serum, saliva, and semen [15,16].

In this study, we first clarified that human hair has a pool of 'oxidized' polysulfide using the EMSP method. Human hair keratin contains a large amount of polysulfide bonds. In addition, we clarified the biological functions of 'oxidized' polysulfide in human hair after treatment with bleaching agents, ultraviolet (UV) radiation, or heat, using antioxidant activity detection methods and electron microscopy.

Materials and methods

Materials

Human hair samples were collected from healthy volunteers between 21 and 39 years old. Written informed consent was obtained from all volunteers prior to the study. The study involving the usage of healthy volunteers' hairs was performed in accordance with the declaration of Helsinki and was approved by the ethics committee of the hospital of Tokushima University (No.3351). Na_2S, Na_2S_2, Na_2S_3, and Na_2S_4 were purchased from DOJINDO Laboratory (Kumamoto, Japan). Ascorbic acid, dimethyl sulfoxide (DMSO), hydrochloric acid (HCl), potassium hydroxide (KOH), iron (III), chloride 5,5-dithiobis-2-nitrobenzoic acid (DTNB), dithiothreitol (DTT), and glutathione were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Hexadecyltrimethylammonium bromide (CTAB) was purchased from the Tokyo Chemical Industry (Tokyo, Japan). Purified human keratin solution and N,N-dimethyl-p-phenylenediamine (DPDA) were purchased from Sigma–Aldrich (Missouri, USA). All chemicals used in this study were of the best grade commercially available, and all solutions were prepared in deionized and distilled water.

Detection of polysulfide using SSP4

Human hair was embedded in an Optimal Cutting Temperature (OCT) compound and kept at −80 °C. These hair samples were cut into 7-μm-thick sections and placed onto glass slides (Matsunami, Osaka, Japan). The human hair sections were incubated with 100 μM SSP4 in 1 mM CTAB + PBS (pH 7.4) solution for 1 h at 25 °C. SSP4-treated hair was washed with PBS three times before obtaining fluorescence images using a fluorescence microscope (model BZ-9000; Keyence, Osaka, Japan) with excitation set at 360 nm and emission at 460 nm.

Quantitative detection of polysulfide in human hair by a modified elimination method of sulfide from polysulfide (modified EMSP)

The polysulfide in human hair was quantified using a modified EMSP method reported previously. Briefly, hair samples (0.5 mg) were incubated with 300 μL of EMSP solution for 24 h at 37 °C. These samples were centrifuged at 2130×*g* for 5 min, and 200 μL of supernatant was collected. A 1 % zinc acetate solution (600 μL) was added to the supernatant and vortexed immediately. The reaction solution samples were centrifuged at 2130×*g* for 5 min and washed with ultrapure water for three times to remove l-ascorbic acid and protein to avoid oxidation by Fe^{3+} in the subsequent methylene blue assay reaction. After removing the supernatants, ultrapure water (200 μL) was added to the precipitates and mixed well. After adding 1 % zinc acetate (300 μL), 50 μL of 20 mM DPDA in 7.2 N HCl, and 50 μL of 30 mM $FeCl_3$ in 1.2 N HCl, followed by vortexing immediately. Then, the solution was incubated for 30 min at 25 °C. Samples were centrifuged at 2130×*g* for 1 min. In the case of purified keratin protein, the keratin was dissolved in a neutral buffer and adjusted to 0.2 mg/ml. The keratin solution reacted with the EMSP buffer. After that, the keratin samples were reacted with the same method as that used for human hair. The supernatants were transferred into 96-well plates, and a plate reader measured the absorbance at 665 nm. Na_2S was used to construct a standard curve. Furthermore, the human hair samples (0.5 mg) were treated with 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (150 μM, 1.0 ml) for 30 min at 25 °C to examine the effect of DPPH treatment on polysulfide in human hair. Then, the polysulfide of the samples was evaluated by modified EMSP.

Oxidation of human hair and purified keratin

Human hair or purified keratin was oxidized by UV irradiation, H_2O_2-treating, or heat treatment. In the case of UV irradiation, human hair or purified keratin was subjected to electromagnetic radiation of 254 nm or 365 nm (UV-C or UV-A) at a 5 cm distance from the sample. The hair samples or purified keratin were enclosed in a 1.5 mL tube (WATSON, Tokyo, Japan). This UV lamp provides a UV intensity of 614 or 743 μW/cm^2 with 254 nm or 365 nm radiation from a distance of 5 cm, respectively. Human hair was exposed to UV for 24 h. The total cumulative amount of UV was 7300 mJ/cm^2 or 8800 mJ/cm^2 with 254 nm or 365 nm. In the case of H_2O_2-treatment, the human hair was bleached with alkaline H_2O_2 (ammonia water: H_2O_2 (28 %): H_2O=1:2:7), respectively, for 0, 1.5, and 6 h [17]. human hair was reacted with H_2O_2 at the same final concentration as bleach (200 mM). After the reaction, human hair was washed three times to remove H_2O_2 using ultrapure water. In the case of heat treatment, the human hair was exposed to heat shock (200 °C, 10 s) using a hair iron (KHC1701VP, KOIZUMI, Osaka, Japan). The hair samples for SEM were heated for 5 min at 180 °C to imitate actual hair iron usage time and temperature. Purified human keratin was heated, respectively, for periods of 0, 3, and 23 h at 100 °C.

Antioxidant analysis by DPPH assay

DPPH (150 μM) in ethanol was mixed with the same volume of ul-

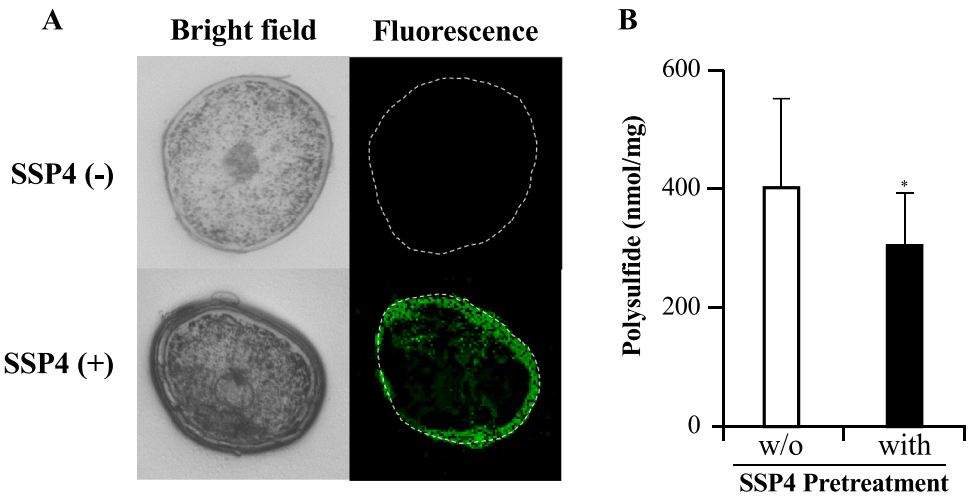

Fig. 1. Detection of polysulfide in human hair sections. (A) Representative photomicrographs of human hair sections treated with 100 μM SSP4 for 1 h. Phase contrast and fluorescence images of polysulfide in human hair sections. Each data shows one representative of three independent experiments using different hair. (B) Detection of polysulfide in human hair using modified EMSP. Human hair was treated with SSP4 as a polysulfide remover. Polysulfide in SSP4-treated or nontreated human hair samples was measured by modified EMSP. The data represent means ± SD ($n = 3$). *P< 0.05 vs. w/o SSP4.

trapure water as a control. Human hair (0.5 mg) or purified keratin (0.5 mg) in ultrapure water was treated with the same amount of DPPH solution for 30 min at 25 °C, and the absorbance of the DPPH radicals was measured at 520 nm. Scavenged radical rates were converted using the following formula:

$$\text{Scavenged radical}(\%) = \left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)/\text{Abs}_{\text{control}} \times 100$$

Human hair samples (0.5 mg) were reacted with 100 μM of SSP4 as a polysulfide remover in 1 mM CTAB/PBS (pH 7.4) for 1 h at 25 °C to clarify the effect of polysulfide in human hair on the antioxidant activity of hair. Then, the antioxidant activity of SSP4-treated hair was measured with a DPPH assay.

Sulfide supply to human hair

Human hair (1 cm) was incubated with 500 μM sodium polysulfides (Na_2S_2, Na_2S_3, and Na_2S_4) in PBS (pH 7.4) for 1 h at 37 °C. After the reaction, human hair was washed to remove excess sodium polysulfides using ultrapure water, and then polysulfide bridges of the human hair were reformed by 0.1 % H_2O_2 for 1 h at 25 °C.

Hair strength test

The strength of a single hair (1 cm of a human hair) was measured with a tension gauge (Ooba, Tokyo, Japan) and slowly pulled at a constant speed (10 cm/min) using a single syringe pump (Fisher brand, PT).

Scanning electron microscopy (SEM)

Human hair samples were fixed on appropriate stubs using carbon double-faced tape. Samples were treated with osmium for 20 min using an osmium coater (Neoc-ST, Meiwaforsis Co., Japan). SEM micrographs were obtained using an SEI detector in a JCM-5700 microscope operating at 20 kV.

Statistical analysis

All values are expressed as the mean ± standard deviation (SD). Two-tailed unpaired t-test or one-way analysis of variance (ANOVA) followed by a Tukey post-hoc test were performed using GraphPad InStat software (GraphPad Software, CA, USA). The levels of significance were set at *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

Result & discussion

Detection of polysulfide in human hair

First, we set out to address whether polysulfide exists in human hair using fluorescent sulfane sulfur probe4, SSP4. Fig. 1A shows that the green fluorescence of SSP4 was observed for the hair samples, suggesting that human hair contains polysulfide. The cuticle was reported to have more cysteine-rich keratin than the medulla [18,19]. SSP4-treated human hair showed an enhanced fluorescence in the cuticle region and at the center of the fiber in the medulla region (Fig. 1A). Furthermore, as shown in Fig. 1, the fluorescence intensity of the cuticle region is stronger than that of the medulla region, indicating that the polysulfide level in the cuticle region is higher than in the medulla region.

Since the SSP4 is unsuitable for quantitative analysis, a different method for quantifying polysulfide in human hair is needed. Therefore, we next attempted to quantify polysulfide in hair using the modified EMSP [14]. This method was developed for polysulfide quantification in liquid samples such as serum, so we optimized the modified EMSP condition to solubilize hair samples. The Fig. S1 shows that the polysulfide in human hair was detected linearly in the range of 0.25 mg/ml to 0.625 mg/ml per sample, and the reaction time with the reducing agent reached a plateau in 24 h (Fig. S1). Using this modified EMSP protocol, we detected approximately 400 nmol/mg polysulfide in human hair, and SSP4 pretreatment significantly decreased polysulfide in human hair. This result suggests that the modified EMSP could quantify polysulfide in human hair, and SSP4 could partially remove polysulfide from the surface of hair (Fig. 1B).

Since the SSP4 results suggest that polysulfides are present on the hair surface, we investigated the presence of polysulfide in the cuticle surface of the human hair using 5-maleimide-fluorescein (5-MF), which reacts with R-SH [20]. Most of the Cys-in human hair was reported to bind with the SH groups of adjacent Cys-and exist as Cys-SS-Cys [21,22]. Furthermore, SH groups on the hair cuticle surface were oxidized [22–25]. Therefore, human hair was first reacted with dithiothreitol (DTT) to generate reduced SH via cleaving the oxidized SS bond before reacting with 5-MF. Moreover, when 5-MF bound with the SnH group, the second DTT treatment reduced the fluorescence intensity (Fig. S2A). Therefore, the presence of SnH groups can be detected by repeating DTT treatment after the 5-MF reaction, to determine whether the 5-MF-bound SH group contains SnH groups. Fig. S2B shows that the fluorescence intensity of 5-MF is decreased by the second DTT

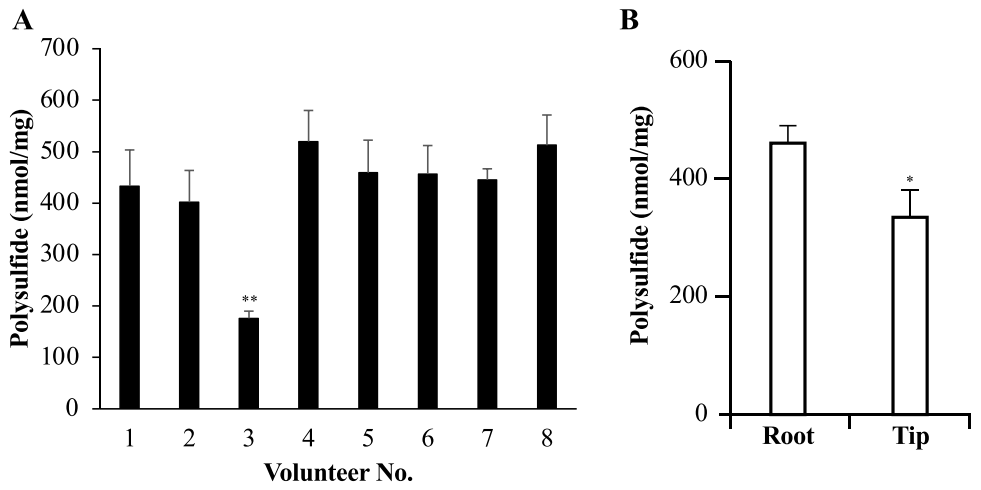

Fig. 2. Quantitative polysulfide detection of human hair from healthy human subjects. (A) Polysulfide in human hair from 8 healthy subjects was measured by modified EMSP. The data represent means ± SD ($n = 3$). (B) Polysulfide in the hair root and tip were measured by the modified EMSP method. The hair data of one of the same experiments performed several times is shown. The data represent means ± SD ($n = 3$). *P< 0.05 vs. Root.

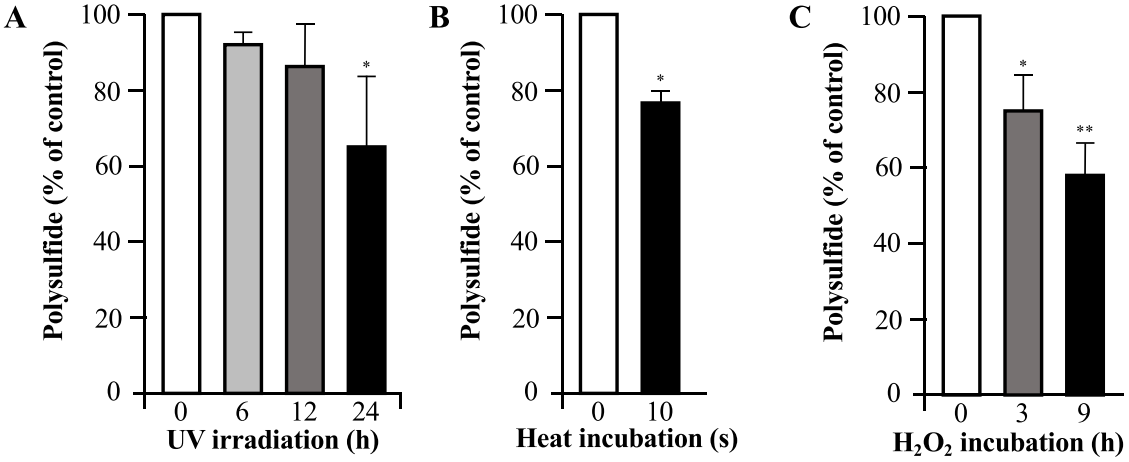

Fig. 3. Effect of various types of oxidative damage on polysulfide in human hair. Human hair samples were exposed to UV (A), heat (B), or H_2O_2 (C) and the modified EMSP method was performed. The hair data of one of the same experiments performed several times is shown. The data represent means ± SD ($n = 3$). *P< 0.05, **P< 0.01 vs. Control or 0 h.

treatment, indicating that polysulfide was present on the surface of the hair cuticle. Considering together with the results of Fig 1A, polysulfide in the hair is abundant in the cuticle and may be present at the junction between cuticles. Therefore, we speculate that polysulfide in the cuticle exists at the outermost layer of the hair, protects the cortex and medulla inside the hair from oxidative stress such as UV, and is related to the beauty of the hair.

Individual differences of polysulfide in human hair

We next focused on the individual differences in hair polysulfide. Disulfide bonds in human hair contribute to the shape and toughness of hair [26,27]. Human hair was collected from 8 subjects, and their polysulfide levels were measured using modified EMSP. Fig. 2A showed individual differences in the hair polysulfide amount of each subject. In particular, the hair of human subject No. 3 with less polysulfide content was probably damaged by repeated hair dyeing and bleaching. These data suggest that hair dyeing and bleaching could affect the polysulfide content of human hair. Previous papers have reported that oxidative stress, such as UV, heat, or chemical treatment with bleaching agents, accumulates at the tip end more than at the root [28]. To clarify the effect of these oxidative stresses on the polysulfide in human hair, we determined the polysulfide at the root and tip of the human hair. The results shown in Fig. 2B indicated that the amount of polysulfide at the tip was significantly lower than that at the root, suggesting that various oxidative stress could affect the polysulfide content in human hair.

Effect of oxidative damage to human hair

Human hair is damaged by the heat of hair dryers, UV, and breaching during daily life activities, and it is known that the degree of damage depends on the individual. Thus, the individual difference in the amount of polysulfide in human hair may be caused by the difference in oxidative stress levels in the human hair during daily activities. Human hair was treated with UV, heat, or H_2O_2 to examine the effect of various oxidation treatments on the polysulfide content of human hair. Fig. 3 shows that UV, heat, and H_2O_2 treatments reduced the amount of polysulfide in the human hair in a time-dependent manner. Therefore, the individual differences in the polysulfide content of human hair may depend on the part of the human hair used for polysulfide measurement and the extent of various oxidative stress exposures such as UV, heat, and bleaching.

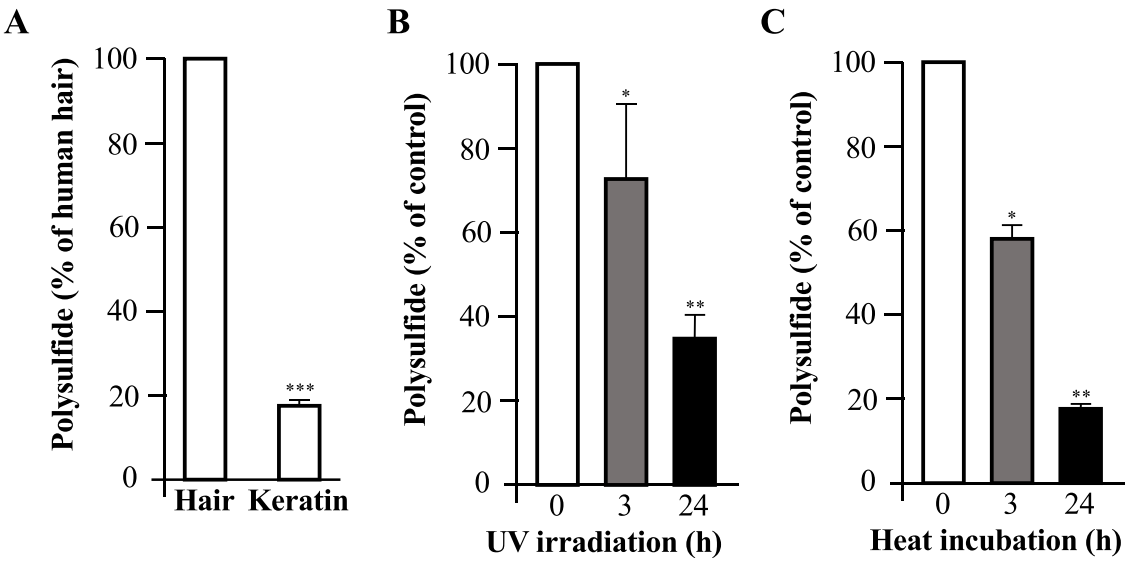

Fig. 4. Effect of various types of oxidative damage on the polysulfide of keratin. Polysulfides of human hair and purified human keratin were detected by modified EMSP (A). Purified keratin was exposed to UV radiation (B) or heat (C), and the modified EMSP method was performed. The data represent means ± SD ($n = 3$). *$P<$ 0.05, **$P<$ 0.01, ***$P<$ 0.001 vs. hair or 0 h.

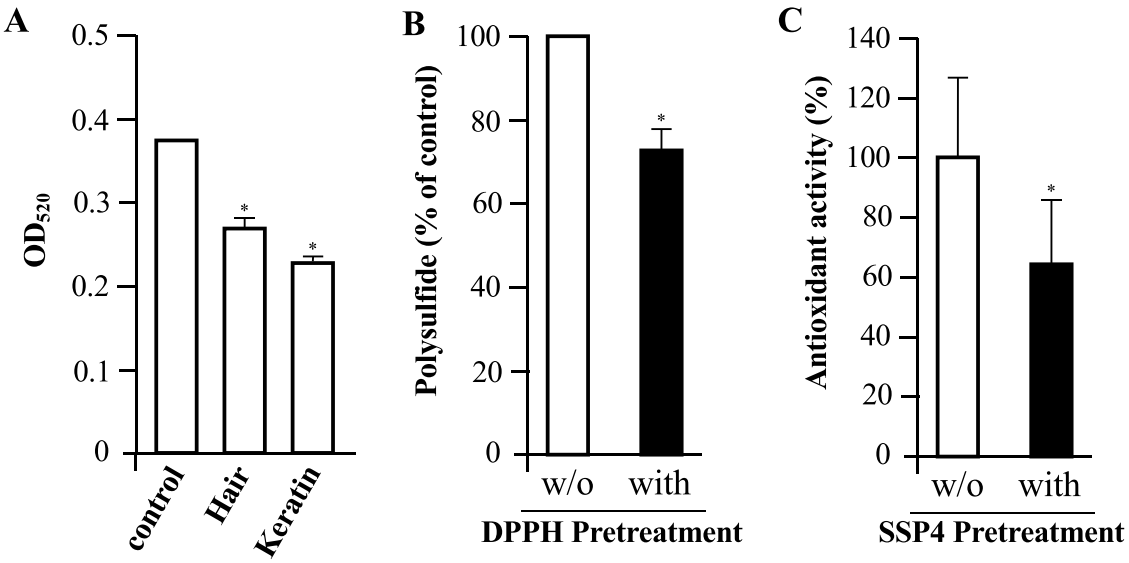

Fig. 5. Antioxidant activity of human hair and keratin. (A) Scavenging of DPPH radical by human hair and purified keratin. The reaction was started by the addition of 1.0 ml solution of 150 μM DPPH solution in ethanol. The reaction mixture was kept at room temperature for 30 min, and the absorbance was measured at 520 nm. (B) The amount of polysulfide in DPPH-treated hair was determined by modified EMSP. (C) The antioxidant activity of hair treated with SSP4 was measured by the DPPH method. The hair data of one of the same experiments performed several times is shown. The data represent means ± SD ($n = 3$). *$P<$ 0.05 vs Control or DPPH or SSP4 (-).

Effect of damage to purified keratin

Keratin is the primary and cysteine-rich protein of human hair [29]. Thus, purified human keratin protein polysulfide was measured with the modified EMSP method to clarify the relationship between polysulfide and keratin protein. Fig. 4A shows that keratin protein possesses approximately 175 nmol/mg polysulfide, approximately 20% of polysulfide in human hair. Reducing agents and alkaline solutions were used in the purification of keratin. Alkaline solutions were used to extract keratin. These factors might have decomposed the polysulfide content of purified human keratin. On the other hand, Fig. 4B and C show that the polysulfide of purified human keratin was decreased by UV or heat treatment, similar to the hair results. These data strongly suggest that the polysulfide of keratin in human hair reacts to oxidation stress.

Antioxidant activity of human hair

According to previously reported results, hydropersulfides (R-SSH) were proposed to be more nucleophilic and superior reducing agents than their other thiol counterparts [30]. For example, GSSH can scavenge H_2O_2 more stronger than GSH because GSSH has more vigorous nucleophilic/antioxidant activity than GSH. Furthermore, GSSSG also has vigorous H_2O_2-scavenging activity. Thus, we hypothesized that polysulfide (Cys-SS_nS-Cys) in human hair might also have antioxidant activity.

Fig. 5A shows that the absorbance of the DPPH radical decreased in the presence of human hair, suggesting that the human hair scavenged the DPPH radical. A similar result was obtained using purified keratin, indicating that human hair and keratin protein have antioxidant

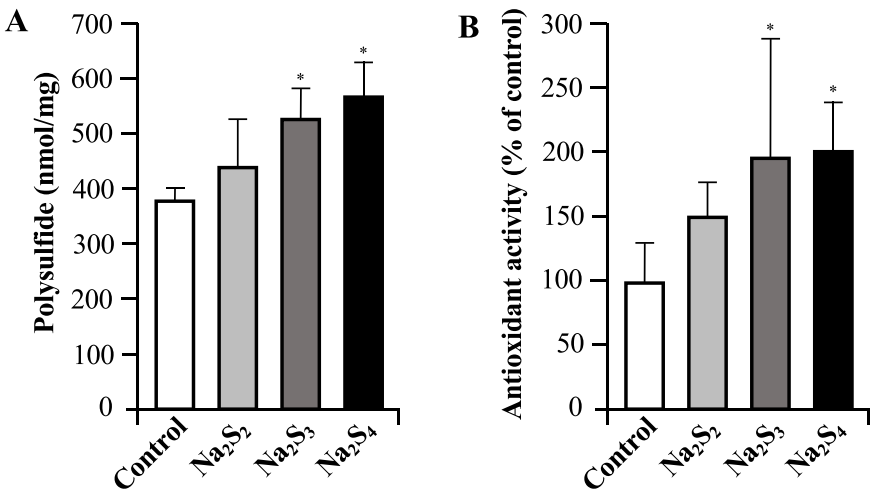

Fig. 6. Sulfide supplied to human hair by incubation with sodium polysulfide. (A) Human hair was treated with sodium polysulfide as a sulfide donor for 1 h. Polysulfide in Na_2S_4-treated human hair samples was measured by modified EMSP after 1 or 2 days passed. (B). Scavenging of DPPH radical by Na_2S_n-treated human hair. The reaction was started by the addition of 1.0 ml solution of 150 μM DPPH solution in ethanol. The reaction mixture was kept at room temperature for 30 min, and the absorbance was measured at 520 nm. The data represent means ± SD ($n = 4$). *P< 0.05 vs. Control.

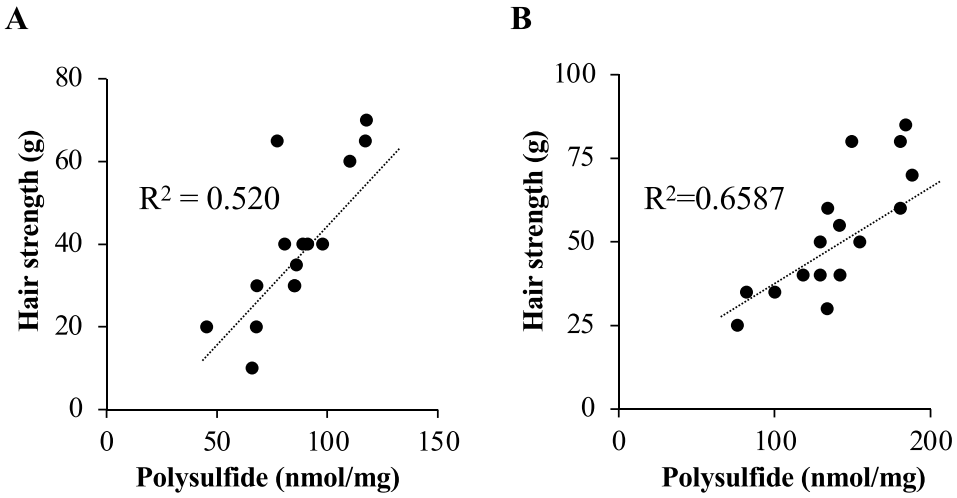

Fig. 7. Relationship between polysulfide in hair and hair strength. After UV irradiation (A) or sulfide supplementation (B), hair strength was measured by strength measuring equipment. Polysulfide in the same sample was measured by a modified EMSP method.

activities. Interestingly, DPPH radical treatment decreased polysulfide in hair compared with control hair (Fig. 5B). Furthermore, the hair antioxidant activity was significantly reduced by SSP4, which removes polysulfide (Fig. 5C). These results strongly indicated that polysulfide in human hair has antioxidant activity.

Supply of sulfide donor to the human hair

Human serum albumin (HSA) has a free thiol at Cys34, which highly reacts with reactive sulfur species. Previously, we reported that sodium polysulfides (Na_2Sn) as polysulfide donors react readily with HSA; the Na_2Sn-treated HSA could efficiently scavenge ROS and NO from chemical reagents [31]. In this study, we investigated the effect of supplying sulfide to human hair via Na_2Sn treatment to enhance the antioxidant activity of human hair. Fig. 6 shows that the amount of polysulfide in the human hair increased depending on the number of sulfurs in the donors and that Na_2S_3 or Na_2S_3 treatments significantly increased the antioxidant activity of the human hair (Fig. 6). This evidence is the first to clarify the protective role of polysulfide in human hair from oxidative stress via the antioxidant ability that can be enhanced by polysulfide supplementation. Additionally, the reaction mechanism between hair and Na_2Sn seem to be different from the reaction between HSA with Na_2Sn. For HSA, its free thiol would react with Na_2Sn to form per/polysulfide. For hair, the proteins do not have free thiol (as shown in Fig. S2). However, the disulfide or trisulfide bonds of hair may also react with Na_2Sn to form per/polysulfides. Future research is necessary to clarify the difference mechanisms.

Relationship between polysulfide in hair and strength

Disulfide bonds in the hair are involved in hair strength, and the cleavage of disulfide bonds reduces hair strength [32]. However, the effect of polysulfide on hair strength has not been demonstrated. To clarify the relationship between human hair strength and the amount of polysulfide in human hair, we performed a human hair strength test and the modified EMSP method. After UV irradiation or sulfide supplementation, hair strength was measured by strength measuring equipment. Fig. 7 showed a positive correlation between the amount of polysulfide and the hair strength in both cases. These findings suggest that polysulfide in human hair may contribute to maintaining hair

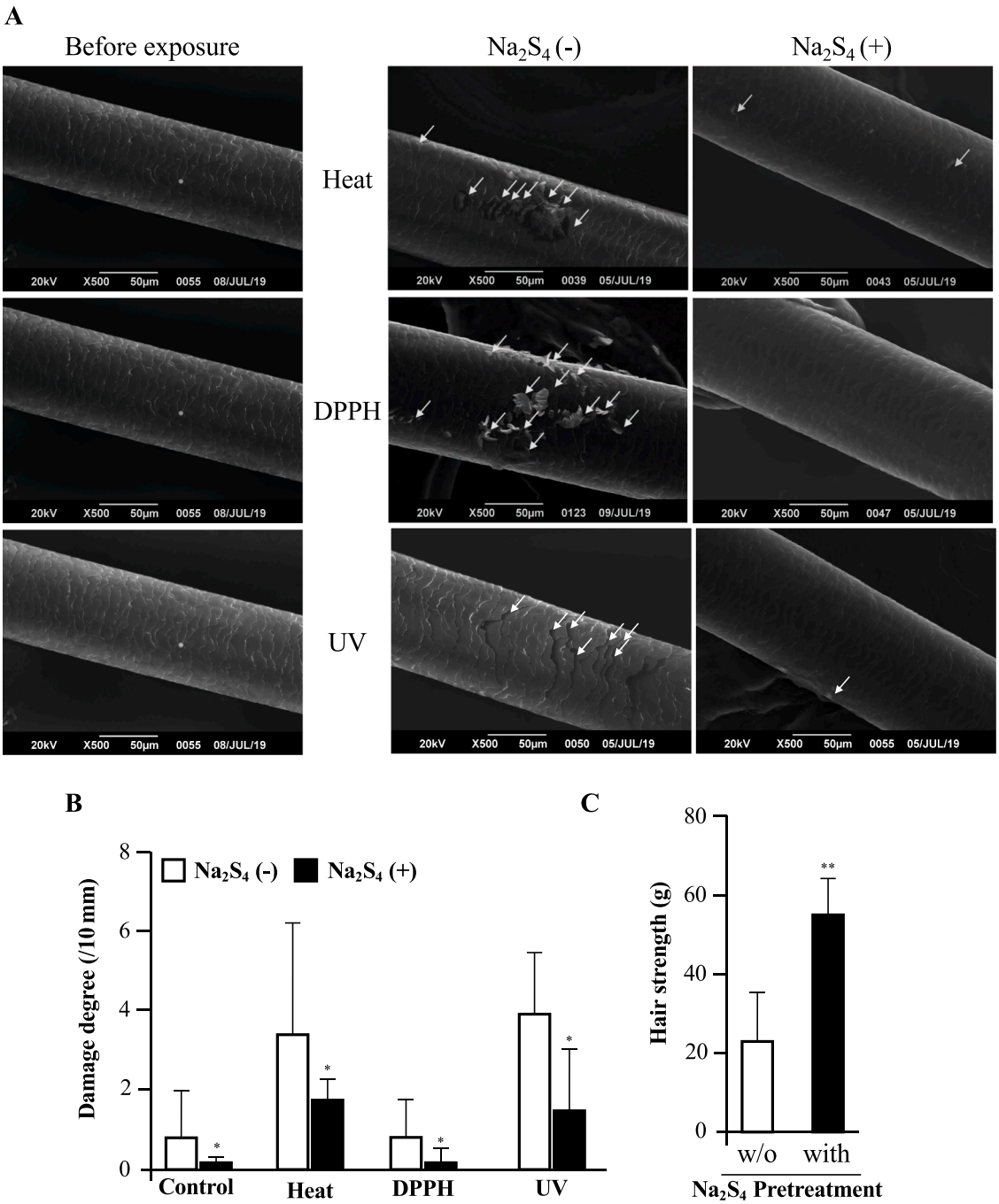

Fig. 8. Protective effect of Na_2S_4 against various oxidative stresses in human hair. (A, B) Sodium polysulfide as a sulfide donor was treated on human hair for 1 h. Na_2S_4-treated human hair samples were exposed to heat, DPPH, and UV irradiation. Coated hair specimens were scanned using an SEM at 15 kV. (C) The treated hair was measured for hair strength. The data represent means ± SD ($n = 4$). *$P< 0.05$, **$P< 0.01$, vs. Na_2S_4 (-) or w/o Na_2S_4 pretreatment.

strength.

Relationship between polysulfide in hair and cuticle

The cuticle is the outer cover of human hair. Furthermore, the cuticle surrounds human hair not by one but several (7 to 10) layers of cuticle cells [33]. Disulfide bonds cross-link the layers of cuticles [34]. Previous reports showed that the barrier function of cuticle layers broke down when the disulfide bonds of cuticles were cleaved in human hair damage [35]. To investigate the relationship between the crosslink of cuticle and polysulfide, we analyzed the human hair using a scanning electron microscope (SEM) after oxidation, such as heat, DPPH, and UV irradiation. All the oxidative stress changed the cuticle structure (Na_2S_n-nontreated hair), while Na_2Sn-pretreated hair decreased cuticle damage (Fig. 8A and B). In addition, the hair strength was increased in Na_2S_4-treated hair (Fig. 8C). These results suggest that the crosslinking of the cuticle involves polysulfide, and that polysulfide contributes to cuticle structure and hair strength.

Conclusion

Keratin of human hair protein is composed of 18 % cysteine that forms disulfide bonds. Cysteine has been reported to be important in maintaining human hair health by protecting hair against oxidative

stress. RSS has higher antioxidant activity than thiols [8]. However, the presence of polysulfide in hair keratin has yet to be clarified. This study demonstrated that polysulfide always exists in human hair, contributing to maintaining the homeostasis of hair. First, polysulfide was detected on the surface of human hair. To clarify the polysulfide distribution of human hair, SSP4 reacted with sulfane sulfur in human hair sections. This result showed that the part of hair cuticle contains sulfane sulfur more than inner of human hair (Fig. 1A), suggesting that human hair could protect against oxidative stress by distributing polysulfides on the cuticle surface. On the other hand, modified EMSP could quantify all polysulfide in whole human hair in order to dissolve human hair by EMSP solution (Fig. 2A). So, we found that there was much polysulfide in hair cuticle and approximately 400 nmol/mg polysulfide. Second, there was an individual difference in the amount of hair polysulfide, and hair polysulfide was significantly decreased by UV, heat, or H_2O_2 treatments. Finally, polysulfide in human hair contributes to hair strength and cuticle attachment strength. A significant finding is that pretreatment of hair with polysulfide donors inhibits hair damage. Therefore, we concluded that polysulfides in human hair have antioxidant activity against oxidative stress and contribute to maintaining hair homeostasis. In the future, it is desirable to develop a hair supplement that increases keratin protein polysulfide with an antioxidative effect and enhances hair strength.

CRediT authorship contribution statement

Takeru Hirai: Conceptualization, Methodology, Writing – review & editing. **Mayumi Ikeda-Imafuku:** Methodology, Writing – original draft. **Nanami Tasaka:** Methodology. **Victor Tuan Giam Chuang:** Writing – review & editing. **Ming Xian:** Methodology, Writing – review & editing. **Tatsuhiro Ishida:** Writing – review & editing. **Takaaki Akaike:** Conceptualization, Methodology, Writing – review & editing. **Yu Ishima:** Conceptualization, Methodology, Funding acquisition, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare no potential conflicts of interest.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.arres.2023.100091.

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