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Implications of Disulfide Bonds in Corneocytes on the Barrier Function of the Stratum Corneum

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This study was conducted to clarify the role of disulfide bonds (SS) in corneocytes on the barrier function and the regulatory mechanism of the formation of SS in the stratum corneum (SC). Trans-epidermal water loss (TEWL) was measured in reconstituted human epidermal models (RHEMs) in which SS levels were modulated by the cleavage of SS with dithiothreitol (DTT). In corneocytes, thiol groups (SH) derived from SS treated with DTT and free SH were separately visualized by labeling with maleimide conjugated to different fluorophores. The oxidation of SH to SS in corneocytes was measured by the decrease in SH or the binding of dansyl glutathione (D-GSH) to corneocytes. Hydrophobicity was estimated by measuring the fluorescence intensity of 1-anilinonaphthalene-8-sulfonic acid (ANS). Quiescin sulfhydryl oxidases (QSOXs) in corneocytes were detected immunochemically. The reduction of SS in corneocytes of RHEMs increased TEWL and concomitantly decreased hydrophobicity. The decreased ratio of SH to SS in corneocytes toward the surface of the skin indicated that the oxidation of SH to SS progresses during cornification. In addition, we consider that QSOX1 could play a role in the oxidation of SH since it was present in corneocytes and the SC. Although SH in corneocytes decreased when incubated in a humidified atmosphere, SH did not decrease when incubated at a relative humidity of 4%. The binding of D-GSH to SH in corneocytes was faster at an acidic pH than at a neutral pH. These results indicated that SS in corneocytes play a crucial role in maintaining the SC barrier, and that SH might be oxidized enzymatically by QSOX1. Furthermore, they showed that a sufficient water content and a pH gradient toward an acidic pH at the surface are required to regulate the oxidation of SH to SS.

Key words: SH group, SS bond, stratum corneum, corneocytes, QSOX

1. Introduction

The stratum corneum (SC), which is composed of corneocytes that are terminally differentiated keratinocytes, and lipid lamellae consisting of ceramides, fatty acids, and cholesterol between the interspaces of corneocytes, is located at the border of the skin with the outer environment and plays a role as a barrier for both inside-out and outside-in protection.[1)] Corneocytes are enclosed within a cornified cell envelope (CCE) composed of various differentiation marker proteins such as envoplakin, periplakin, involucrin and loricrin, which are cross-linked by transglutaminase-1 (TGM-1),

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instead of a plasma membrane.[2] The inside of each corneocyte is filled with keratin filaments aggregated by filaggrin. Furthermore, to serve as a scaffold for the formation of lipid lamellae and to acquire hydrophobic properties, each corneocyte possesses a cornified lipid envelope (CLE). The CLE is formed by ω-hydroxyceramide and esterified ceramide, where linoleic acid is covalently bonded to the ω-hydroxy acyl group of ceramides.[3] Considering their contribution to the barrier function of the skin, corneocytes might control the barrier due to their rigid structure, similar to lipid lamellae. Thus, it is considered important to produce normal corneocytes during terminal differentiation and cornification to acquire an effective barrier function.

Recently, we reported that the ratio of free thiol groups (SH) to disulfide bonds (SS) (SH:SS) is higher in dry skin, which is characterized by a low skin surface water content and a high trans-epidermal water loss (TEWL).[4] While SH is abundant in the layers of living cells in the epidermis, SS are observed in the SC in accordance with the disappearance of SH. As it is known that loricrin has a high amount of cysteine residues,[5] the higher SH:SS ratio may suggest the incomplete formation of the CCE. However, despite the ratio of SH to SS being a parameter indicating skin dryness, there are few studies that have assessed the role of SS in corneocytes in relation to skin moisture.

Generally, the enzymatic oxidation of SH to SS is carried out by quiescin sulfhydryl oxidase (QSOX), which is a flavin-linked SOX.[6] Indeed, it has been reported that QSOX1 and QSOX2 exist in the epidermis and in hair follicles.[7] If the oxidation of SH to SS occurs gradually in corneocytes during cornification, it is presumed that QSOXs present in the SC oxidize the SH residues of proteins in corneocytes to SS. On the other hand, since SH is unstable against oxidation, SH can also be chemically oxidized to SS. Therefore, the presence of both enzymatic and chemical oxidation makes it difficult to prove the oxidation pathway of SH in the SC.

Thus, this study was conducted to clarify the implications of SS in corneocytes on the SC barrier function and to elucidate factors that regulate the oxidation of SH to SS.

2. Materials and Methods

2.1. Reagents

1-Anilinonaphthalene-8-sulfonic Acid (ANS) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (Darmstadt, Germany). $CaCl_2$, $MgCl_2$, KBr, NaCl, and KCl were purchased from Kanto Chemical (Tokyo, Japan). Chloroform ($CHCl_3$), methanol (CH_3OH), dithiothreitol (DTT), Tris (hydroxymethyl) aminomethane (Tris), and acetic acid were purchased from Nacalai Tesque (Kyoto, Japan). Dansyl glutathione (D-GSH) was purchased from Watanabe Chemical Industries (Hiroshima, Japan). DyLight 633 maleimide was purchased from ThermoFisher Scientific (Waltham, MA, USA). *N*-(7-dimethylamino-4-methylcoumarin-3-yl) maleimide (DACM) was purchased from FUJIFILM Wako Chemicals (Osaka, Japan). The anti-QSOX1 antibody (12713-AP) was purchased from Proteintech (Rosemont, IL, USA), and the QSOX2 antibody (ab121376) was purchased from Abcam (Cambridge, UK). The anti-rabbit antibody conjugated to Alexa Fluor 594 and the anti-rabbit antibody conjugated to horseradish peroxidase (HRP) were purchased from Cell Signaling (Danvers, MA, USA) and ThermoFisher Scientific (Darmstadt, Germany), respectively. ImmPACT DAB Substrate Kit Peroxidase was purchased from Vector Laboratories (Burlingame, CA, USA).

2.2. Reconstructed human epidermal models (RHEMs)

LabCyte EPI-MODELs (RHEMS) were purchased from J-TEK (Aichi, Japan). To obtain the SC, RHEMs were fully cornified by continuing their culture in a specific medium (J-TEK) at 37°C in a 5% CO_2 atmosphere for 4 weeks. The medium was changed every 3 days with fresh medium.

2.3. Corneocytes

The collection of corneocytes from volunteers and their use for this study were approved by the CIEL Ethical Committee (Approval number of testing: C-2022001). After explaining the study protocol, volunteers who agreed to provide corneocytes for this study signed a written informed consent. Corneocytes were collected from the inside of the forearm of each volunteer (4 females, 1 male, ages ranging from 30 to 65 years) using cellophane tape (Nichiban, Tokyo, Japan) or KAKUSHITSU checkers (ASCH Japan, Tokyo, Japan). Corneocytes in the third layers stripped with KAKUSHITSU checkers were used for subsequent examinations. Furthermore, a portion of each KAKUSHITSU checker containing corneocytes was stored at −20°C to determine the level of SH as a baseline. On the other hand, corneocytes collected by stripping with cellophane tape were used for examinations after being transferred onto MAS coat glass slides (Matsunami, Osaka, Japan) as follows: The glass slides were tightly pressed to the cellophane tape with the collected corneocytes and were immersed in xylene to remove the cellophane, after which they were immersed in fresh xylene to completely remove the adhesive agent of the cellophane tape.

2.4. Cleavage of SS to SH with DTT

The cleavage of SS to SH in RHEMs and in tape-stripped corneocytes was carried out by incubation with DTT as follows: RHEMs were covered with a 100 mM Tris-acetate-saline (TAS)-buffered solution (pH 6.8) containing 1 or 10 mM DTT and were then incubated at 37°C for 24 h while soaking the bottom of the RHEM in phosphate-buffered saline (PBS) at 37°C. Corneocytes transferred to glass slides were incubated with a 100 mM TAS-buffered solution (pH 6.8) containing different concentrations of DTT for 2 h at 37°C.

2.5. Measurement of TEWL

TEWL values of RHEMs, in which SS were cleaved, were measured using a VAPOSCAN AS-VT100RS (ACSH Japan, Tokyo, Japan) after adjusting the temperature to 37°C by soaking the bottom of the RHEM in PBS at 37°C.

2.6. Thin sections of RHEMs

Each RHEM was embedded in O.C.T. Compound (Sakura Finetek, Tokyo, Japan) and was frozen in liquid nitrogen. Frozen sections of 6 μm thickness were prepared using a cryostat Leica CM1520 (Leica Biosystems, Hessen, Germany).

2.7. Fluorescence labeling of SH

Fluorescence labeling of SH in thin sections of RHEMs and in corneocytes was performed by incubation with a 100 mM TAS-buffered solution (pH 6.8) containing 10 μM DACM or 2 μg/mL DyLight 633 maleimide at 37°C for 2 h in the dark.

2.8. Treatment with ANS

Thin sections of RHEMs and corneocytes were incubated with a 100 μM ANS aqueous solution for 15 min at room temperature after washing 2 times with $CHCl_3$:CH_3OH (2:1).

2.9. Immunostaining of QSOX1 and QSOX2 in human skin and in corneocytes

Human skin was purchased from Biopredic (Rennes, France). Thin sections of human skin were fixed with 4% paraformaldehyde and corneocytes were incubated with the anti-QSOX1 antibody (1:200) or the QSOX2 antibody (1:200) for 18 h at 4°C after blocking with PBS containing 1% BSA. After washing with PBS containing 0.05% Tween-20, corneocytes were incubated with the anti-rabbit antibody conjugated to Alexa Fluor 594 (1:250) at room temperature. Thin sections of human skin were incubated with the anti-rabbit antibody conjugated to HRP (1:250) for 2 h at room temperature in the dark, and were colorized with ImmPACT DAB Substrate Kit Peroxidase.

2.10. Binding of D-GSH to SH in corneocytes

Corneocytes were incubated in buffered solutions that were adjusted to various pH levels and contained D-GSH for 3 h at 37°C.

2.11. Fluorescence imaging and the quantification of fluorescence

Fluorescence images of corneocytes and thin sections of RHEMs were obtained using an All-in-One fluorescence microscope BZ-X810 (KEYENCE, Osaka, Japan). The fluorescence intensity was quantified using specialized software, corneocytometry 2 (CIEL, Tokyo, Japan).

2.12. Statistics

All data are expressed as means ± SD. Comparisons between 2 groups were performed using Student's t-test. Correlation coefficients of data were analyzed by Pearson's correlation coefficient. A p value < 0.05 is considered statistically significant.

3. Results

3.1. Role of SS in corneocytes

To clarify the role of SS in corneocytes on the barrier function, we measured TEWL in RHEMs after cleaving SS to SH with DTT. Although no significant difference was found between 1 and 10 mM DTT, RHEMs treated with DTT showed a significantly higher TEWL value, which correlated with an increased level of SH (Figs. 1A, 1B). Concomitantly, the hydrophobicity of RHEMs was measured using the fluorescence intensity of ANS. Changes in fluorescence intensity derived from ANS are generally used to measure hydrophobicity in microdomains, with increased fluorescence corresponding to increased hydrophobicity.[8] Furthermore, we have also reported that the hydrophobicity of hair and corneocytes can be measured using the ANS fluorescence.[9,10] The fluorescence intensity of ANS in RHEM sections decreased with increased level of SH (Fig. 1C). Additionally, the fluorescence intensities of ANS in corneocytes showed a negative correlation with increased SH levels in the same corneocytes ($p < 0.05$) (Fig. 1D). These results indicated that SS in corneocytes contribute to maintaining the SC barrier and also the hydrophobic properties of corneocytes in the SC.

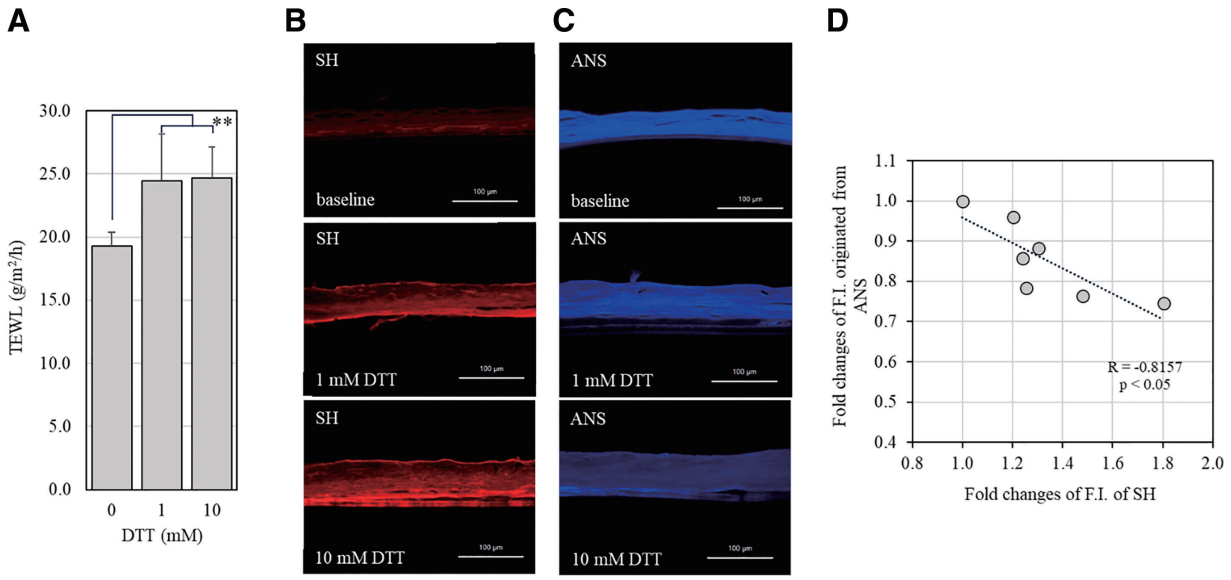

Fig. 1 TEWL and hydrophobicity in RHEMs treated with DTT. RHEMs were cultured for 4 weeks to obtain the SC. TEWL values of the SC treated with DTT on the surface were measured using a Vaposcan AS-VT100RS. The level of SH in frozen thin sections of RHEMs was measured using DyLight 633 maleimide. After de-lipidation, thin sections were treated with ANS. (A) TEWL values of RHEMs are presented as means ± SD, n = 4. Significance ** $p < 0.01$. (B) Representative images of SH in the SC of fluorescence-labeled RHEMs. Scale bars: 100 μm. (C) Representative fluorescence images of ANS. Scale bars: 100 μm. (D) Correlation between the fluorescence of corneocytes that originates from ANS and the level of free SH in corneocytes. The experiment was carried out in triplicate.

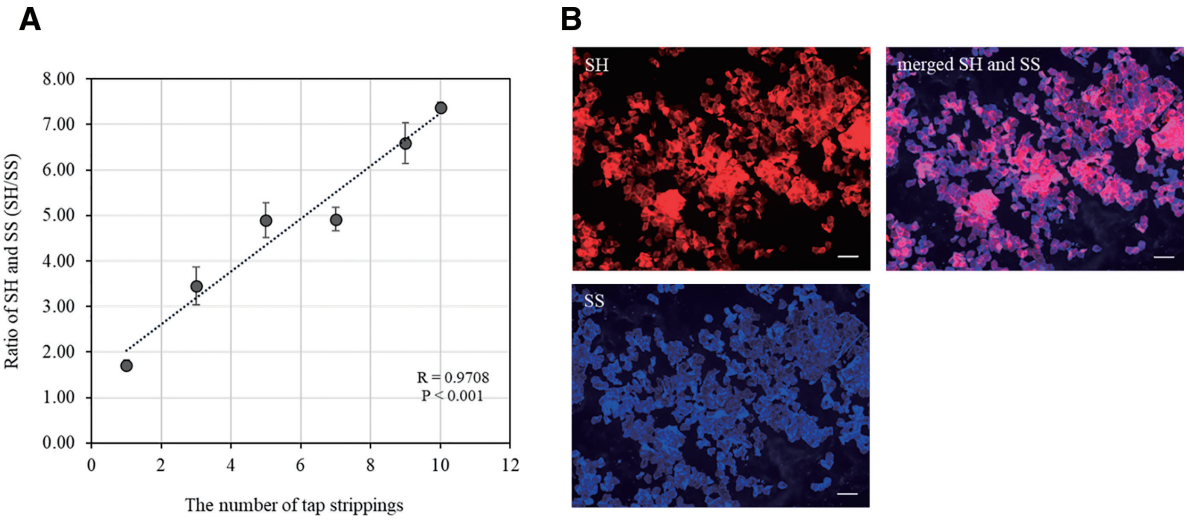

Fig. 2 Changes of SH:SS in the SC depending on the number of tape strippings. SH groups and SS bonds in corneocytes obtained by tape stripping from 3 volunteers were visualized by fluorescence labeling. SH groups were labeled with 2 μg/mL DyLight 633 maleimide. After cleaving SS bonds with 400 μM DTT, SH that originated from SS was labeled with 10 μM DACM. (A) Ratios of SH and SS of corneocytes tape-stripped in each layer of the SC. (B) Typical images of SH and SS in corneocytes when SH and SS were double-labeled. Scale bars: 100 μm.

3.2. SH and SS in corneocytes during cornification

The ratio of SH to SS (SH:SS) in corneocytes at different depths of the SC was investigated. Since the SH:SS ratio showed a positive correlation with the increased number of tape strippings, it indicated that SH is gradually oxidized to SS as the corneocytes migrate toward the surface of the SC (Figs. 2A, 2B).

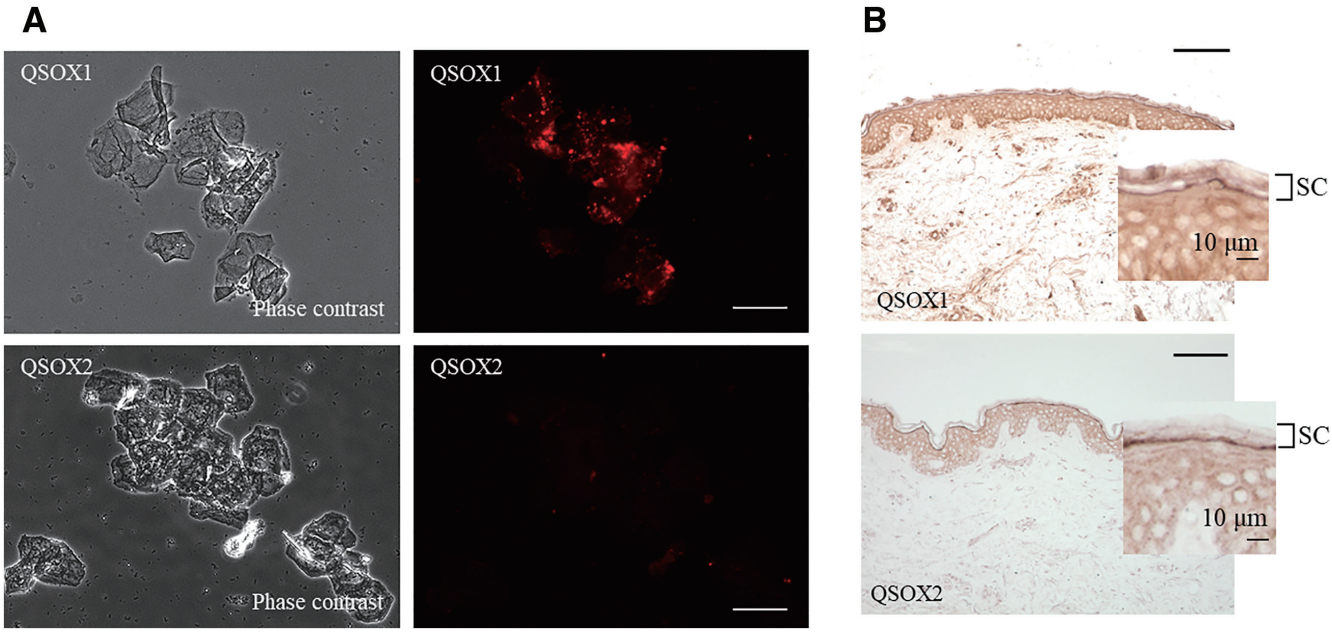

Fig. 3 Expression of QSOX1 and QSOX2 in corneocytes and in the epidermis. QSOX1 and QSOX2 in corneocytes and in human skin were immunostained with specific antibodies. (A) Representative images of QSOX1 and QSOX2 in corneocytes. Scale bars: 100 μm. (B) Representative images of QSOX1 and QSOX2 in human epidermis.

3.3. QSOX levels in corneocytes and in the SC

The distribution of QSOX1 and QSOX2 in the epidermis was investigated using immunostaining. The QSOX2 signal was almost undetectable in corneocytes and the SC, despite its expression in living cell layers (Figs. 3A, 3B). On the other hand, the QSOX1 signal was detected throughout the entire epidermis, including corneocytes. These results indicated that QSOX1 predominantly exists in the SC, suggesting that it might play a role in the oxidation of SH in the SC.

3.4. Oxidation of SH to SS in corneocytes under a humidified atmosphere

Corneocytes collected immediately after collection by the KAKUSHITSU checker were incubated in a humidified atmosphere at 37°C to examine the oxidation of SH. The level of SH in corneocytes decreased in a time-dependent manner during incubation in a humidified atmosphere at 37°C (Fig. 4A), and the decrease in SH in corneocytes was restored to the baseline by treatment with DTT (Fig. 4B). These results indicated that SH residues in corneocytes were oxidized to SS during incubation in a humidified atmosphere at 37°C.

3.5. Impact of relative humidity on the oxidation of SH in corneocytes

We examined the influence of environmental humidity on the oxidation of SH in corneocytes. Atmospheres with different relative humidities (RHs) were prepared using saturated aqueous solutions of inorganic salts. Corneocytes showed sharply decreased levels of SH at RHs above 61%, while a RH of less than 31% had less decrease in SH. Additionally, a RH of 4% had no significant effect on the level of SH in baseline corneocytes (Fig. 5A). Since the decrease in SH was restored to the level of SH in baseline corneocytes by treatment with DTT, it confirmed that the decreases were due to oxidation to SS (Fig. 5B).

3.6. Influence of pH on the oxidation of SH in corneocytes

The influence of pH on the oxidation of SH in corneocytes was investigated by measuring the decrease in SH in corneocytes and the binding of D-GSH as a fluorescent substrate for disulfide bonding to corneocytes. In a preliminary study, we confirmed that the fluorescence derived from D-GSH was present in corneocytes, and that it disappeared upon treatment with DTT (data not shown). These facts indicated that D-GSH is useful for measuring the formation of SS in corneocytes as a fluorescent probe. A buffered solution at pH 4.5 showed a significant decrease in SH in corneocytes, whereas a buffered solution at pH 7.4 showed a gradual decrease in SH in corneocytes (Fig. 6A). Furthermore, the results showed that there is a similar pH dependence in the binding of D-GSH to corneocytes (Figs. 6B, 6C). These results indicate that the oxidation of SH to SS in corneocytes is modulated by pH conditions.

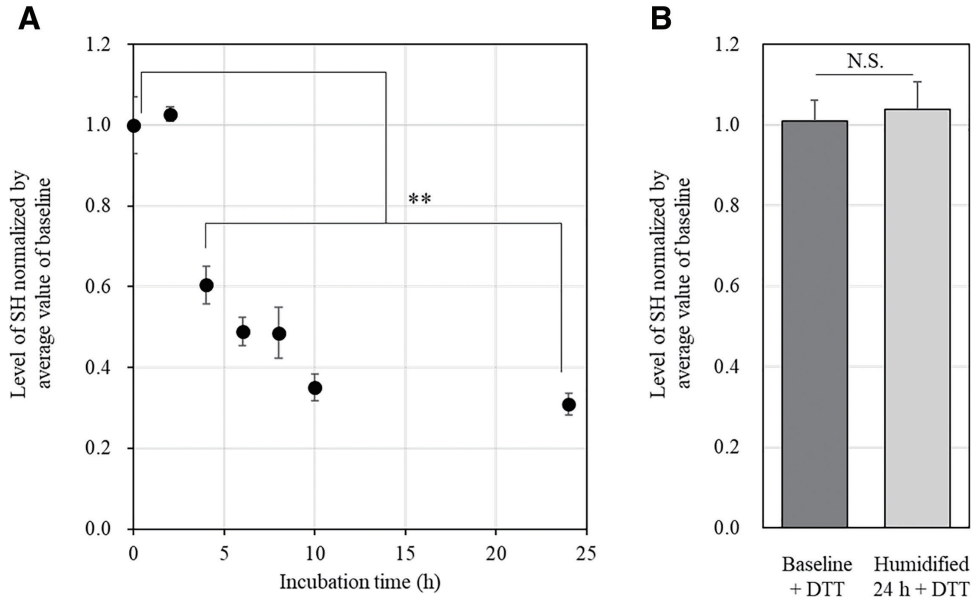

Fig. 4 Oxidation of SH to SS in corneocytes in a humidified atmosphere. Corneocytes were incubated in humidified conditions (RH: 92%) at 37°C for 24 h (humidified) or were stored at −20°C (baseline). SH in corneocytes was visualized by fluorescence labeling with DyLight 633 maleimide. Levels of SH in corneocytes are expressed as values normalized by the average value of the baseline. The experiment was carried out in triplicate. (A) Time-dependent changes of SH in corneocytes. (B) To determine whether the change of SH in corneocytes originates from its oxidation to SS, baseline and humidified corneocytes were treated with 400 μM DTT. The level of SH in corneocytes is expressed as a value normalized by the average value of the baseline or of the baseline treated with DTT. Significance: $^{**}p < 0.01$ (Dunnet's t-test).

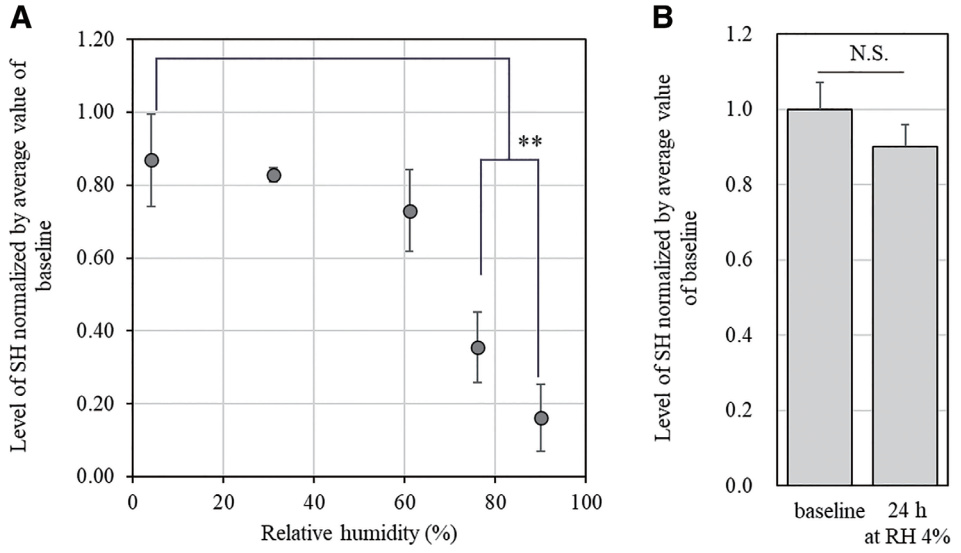

Fig. 5 Changes of SH in corneocytes incubated under atmospheres at different RHs. Corneocytes were incubated under different humidified conditions at 37°C for 24 h or were stored at −20°C (baseline). Each humidified condition was prepared with a saturated inorganic salt solution; a RH of 4% with $CaCl_2$, a RH of 31% with $MgCl_2$, a RH of 61% with KBr, a RH of 76% with NaCl, and a RH of 90% with KCl. SH in corneocytes was visualized by fluorescence labeling with DyLight 633 maleimide. Levels of SH in corneocytes are expressed as values normalized by the average value of the baseline. The experiments were carried out in triplicate. (A) Changes of SH in corneocytes incubated at different humidified conditions. (B) To determine whether the change of SH in corneocytes originates from oxidation to SS, baseline and humidified corneocytes were treated with 400 μM DTT. Levels of SH in corneocytes are expressed as values normalized by the average value of the baseline or of the baseline treated with DTT. Significance: $^{**}p < 0.01$ (Tukey–Kramer's t-test).

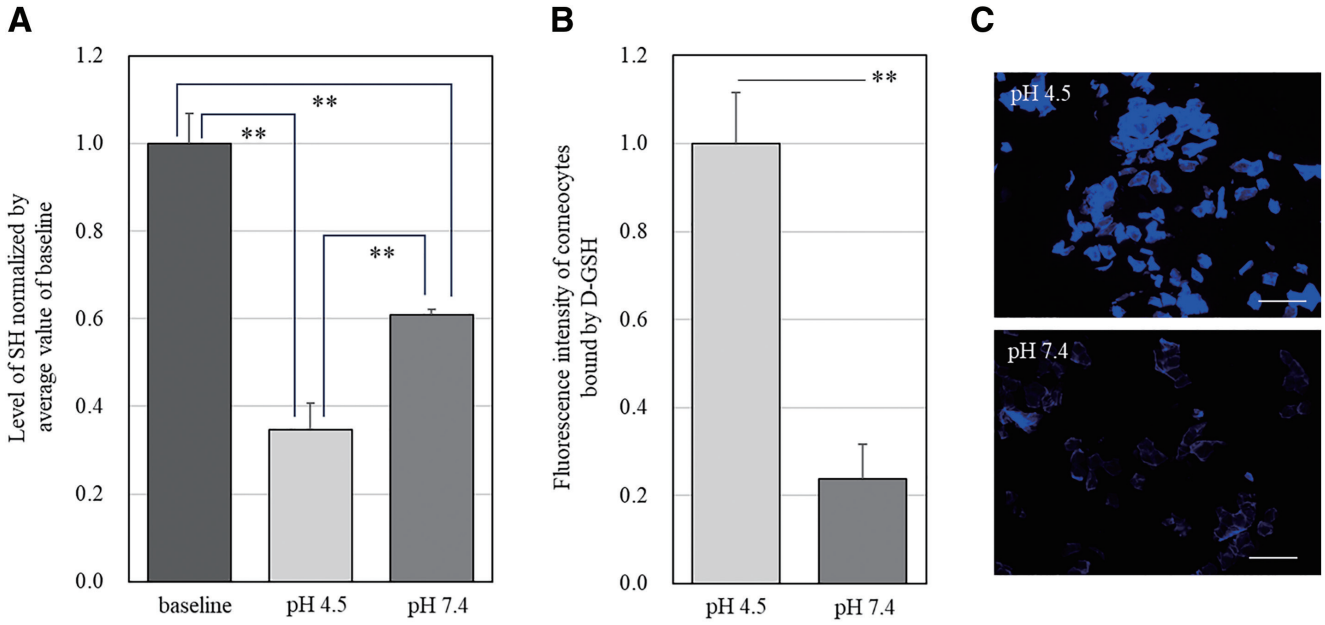

Fig. 6 Changes of SH in corneocytes and bonding of D-GSH to SH in corneocytes incubated in different pHs.
(A) Corneocytes tape-stripped with KAKUSHITSU checkers 3 times were incubated in different pH conditions at 37°C for 24 h or were stored at −20°C (baseline). SH in corneocytes was visualized by fluorescence labeling with DyLight 633 maleimide. Levels of SH in corneocytes are expressed as values normalized by the average value of the baseline. The experiments were carried out in triplicate. Significance: $^{**}p < 0.01$ (Tukey–Kramer's test). (B) Corneocytes tape-stripped with KAKUSHITSU checkers 3 times were incubated with 200 μM D-GSH in buffered solutions having different pHs at 37°C for 3 h. The bonding of D-GSH to corneocytes was detected by measuring the fluorescence that originated from the dansyl group. The degree of bonding of D-GSH is expressed as a value normalized by the average value of corneocytes incubated in a buffered solution at pH 4.5. The experiments were carried out in triplicate. Significance: $^{**}p < 0.01$ (Student t-test). (C) Representative fluorescence images of corneocytes incubated with D-GSH at different pHs. Scale bars: 100 μm.

4. Discussion

In a previous epidemiological study, we reported that the ratio of SH to SS (SH:SS) in corneocytes is increased in dry skin, which is characterized by lower values of skin surface water content and higher values of TEWL.[4] In general, SH is present at high levels in living cell layers of the epidermis, and SS are increased in the SC.[11] In fact, in this study, it was also found that the ratio of SH to SS in corneocytes decreased toward the surface of the skin (Fig. 2), which indicates that the formation of SS progresses with the maturation of corneocytes. Considering these findings, there are 2 perspectives regarding the significance of a high ratio of SH to SS in dry skin: whether the high frequency of SH remaining on the skin surface is merely a phenomenon resulting from dry skin or whether the remaining SH affects the progression or maintenance of dry skin. However, despite the fact that the ratio of SH to SS is a parameter related to the maturation of corneocytes and to skin dryness, to date, the role of SS in corneocytes on their function regarding skin moisture has not been determined precisely. Thus, the first purpose of this study was to clarify the role of SS, focusing on the SC barrier.

Although the SC barrier consists of intercellular lipid lamellae structures and corneocytes, only corneocytes are the solid components responsible for the barrier function. Thus, it is expected that corneocytes function as a physical shield against substances moving from the inside-out or from the outside-in, for example, water. Thus, to clarify the initial purpose of this study, we examined the TEWL value of the SC in RHEMs, where the level of SH was modulated by reducing SS in corneocytes with DTT. The results showed that TEWL values were elevated in accordance with the increase in SH in corneocytes (Figs. 1A, 1B). These results suggest that SS in corneocytes are critical for the SC to function as a barrier against water. Simply thinking of corneocytes functioning as a barrier, one of the requirements is hydrophobicity, which is achieved through the dense structure of corneocyte proteins. Thus, we measured the hydrophobicity of the SC of RHEMs. The fluorescence intensities of the SC treated with ANS declined with elevations of TEWL and SH

(Fig. 1C). Furthermore, the fluorescence intensity of corneocytes treated with ANS was negatively correlated with levels of SH in those corneocytes (Fig. 1D). The sum of these results indicates that SS in corneocytes are important for acquiring a hydrophobic SC and for playing a role in the function of the SC as a barrier. Considering the structure of corneocytes, the inside of the CCE is filled with keratin fibers composed of keratin 1 (K1) and keratin 10 (K10). Since K1 and K10, which are soft keratins, have a lower ratio of cysteine residues,[12] it was not expected that they would contribute to acquiring the hydrophobic property through the formation of SS. On the other hand, the CCE has a densely packed protein structure through *N*-ε(γ-glutamyl) lysine linkages produced by transglutaminase-1 and is regarded as contributing to the hydrophobicity of corneocytes.[2] In structural proteins of the CCE, it is presumed that loricrin, which accounts for approximately 80% of the CCE, plays a predominant role in the strength of the CCE through the formation of SS and provides the hydrophobic property to corneocytes.[5] When considered in this way, the importance of SS in the CCE is suggested to generate and maintain the SC barrier.

However, despite the importance of SS in corneocytes, little is known about how the oxidation of SH to SS is regulated within the SC. Indeed, we found that QSOX1 is present in the SC and in corneocytes (Fig. 3), and further, that corneocytes retain the ability to oxidize SH to SS when incubated in a humidified atmosphere (Fig. 4) and lose that ability in a dry atmosphere (Fig. 5). Generally speaking, the oxidation of SH to SS is carried out through enzymatic and chemical mechanisms due to the instability of SH against oxidation. Thus, although it is challenging to demonstrate these oxidation mechanisms separately, the results indicated that the presence of adequate amounts of water is required for both oxidation mechanisms and suggested that the enzymatic formation of SS in corneocytes is presumably carried out by QSOX1. However, it is difficult to explain that the oxidation of SH to SS within the SC is solely regulated by water because, despite the higher amount of water in deeper layers of the SC compared to the surface layers, SH is highly retained in the deeper layers. Thus, there must be another regulatory factor to maintain SH in deeper layers of the SC.

Finally, we considered the potential involvement of pH as another regulatory factor in the oxidation of SH to SS. It is known that the SC has a pH gradient from neutral to acidic toward the surface of the skin.[13] The oxidation of SH in corneocytes occurred more rapidly at an acidic pH (pH 4.5) compared to a neutral pH (pH 7.4), as evidenced by the decrease in SH in corneocytes and the increase in fluorescence due to the binding of D-GSH to corneocytes (Fig. 6). Thus, considering pH as another regulatory factor, the distribution of SH and SS within the SC at a specific depth can be explained. However, we currently do not have any evidence about how an acidic pH regulates the enzymatic and chemical oxidation of SH to SS. As the next step, we will investigate the regulatory mechanism on SH oxidation regarding pH.

5. Conclusion

The results of this study indicate the importance of the oxidation of SH to SS in corneocytes by showing that SS play a role in maintaining the SC barrier and hydrophobicity due to the linkage of proteins in corneocytes. Additionally, the results indicate that water is an essential factor and that pH is a regulatory factor in the oxidation of SH to SS in corneocytes. To maintain the SC barrier through the proper oxidation of SH to SS, the results suggest the importance of maintaining proper water content and a pH gradient in the SC.

Conflicts of interest: There are no conflicts of interest.

Abbreviations: ANS, 1-anilinonaphthalene-8-sulfonic acid; BSA, bovine serum albumin; CCE, cornified cell envelope; CLE, cornified lipid envelope; $CHCl_3$, chloroform; CH_3OH, methanol; D-GSH, dansyl glutathione; DACM, N,-(7-dimethylamino-4-methylcoumarin-3-yl) maleimide; DTT, dithiothreitol; HRP, horseradish peroxidase; K1, keratin 1; K10, keratin 10; PBS, phosphate-buffered saline; QSOX, quiescin sulfhydryl oxidase; RH, relative humidity; RHEM, reconstituted human epidermal model; SC, stratum corneum; SH, thiol groups; SS, disulfide bonds; TAS, Tris-acetate-saline; TEWL, trans-epidermal water loss; TGM-1, transglutaminase-1; Tris, Tris (hydroxymethyl) aminomethane

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