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Visual observation of selective elution of components from skin-mimetic lipid membrane

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ABSTRACT

Selective elution of components was visually observed on a mixture of lipids (ceramide III, palmitic acid, and cholesterol) as a mimicry of stratum corneum (SC) which was melted and sandwiched between glass plates. The lipid membrane was exposed to an aqueous solution of sodium dodecyl sulphate (SDS) and observed by an optical microscope. The contact of the lipid membrane with a SDS solution caused the elution of the lipid component as "myelin-form", and the lipid membrane changed to a sponge structure. An infrared absorption spectroscopic study revealed that the SDS penetrated into the lipid mixture, and the fraction of ceramide in the sponge phase became higher than that in the lipid membrane before SDS treatment. The selective elution behaviour was confirmed by observing the behaviour of each component in lipid membrane by means of a fluorescence-staining method: The cholesterol was eluted with producing visual myelin-form on the contact with a SDS solution, and the following elution of palmitic acid occurred without myelin-form, while the ceramide III resisted the exposure to the SDS solution. These results are valid to elucidate the influence of surfactants on SC.

1. Introduction

Stratum corneum (SC) is the outmost layer of animal skin and regarded as a barrier against penetration and release of substances. It takes a brick-and-mortar structure consisting of corneocyte keratin and intercellular lipids [1,2]. The lipids in human SC mainly consist of ceramides, fatty acids and cholesterols. They form lamella between the corneocytes, where the lipid molecules are arranged with ordered packing [3]. Such structure is considered to contribute to the barrier function [4–6], and its disordering is associated with impairment of skin [7–9].

As a trigger of the disordering in lamella structure, interactions with surfactants have been attracted attention in dermatology [4–9]. Researches indicate that surfactants can penetrate the SC and release the lipids from the SC. Using native and mimic SCs, it has been shown that SC profiles would be significantly changed after exposure to surfactants [10–14]. Although the diminution of barrier function was confirmed as a result of surfactant treatment, the mechanism of surfactant effects remains relatively obscure. Structural analyses of the lipids in SC have been performed with native and mimic SC to realize the role of lipids [15–18], but further research is required to combine the fundamental characteristics of lipids with their peculiar properties. It has been reported in terms of morphology that lipids change their organization from lamella to vesicles in the SC after surfactant treatment [19]. Although this structural transition is a direct evidence of disordering in intercellular lipids, the mechanism of this change is not necessarily clarified. On the accurate interpretation of this phenomenon, the effect of surfactants on SC must become more obvious, and effective ways to prevent the damage of skin from the surfactants must be developed.

The present study focuses on the morphological change during the treatment by surfactant on SC. Using mimetic human SC consisting of ceramide, palmitic acid and cholesterol, when mimetic lipid membrane is exposed on an aqueous solution of sodium dodecyl sulphate (SDS), the variating process of the membrane is observed by optical and fluorescent microscopic techniques. The results will bring the breakthrough on the dermatological insight for damage and repair of human skin.

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2. Experimental

2.1. Materials

Ceramide III (95.9%) was supplied from Evonic Degussa (Essen, Germany). Palmitic acid (95.0%) and cholesterol (guarantee reagent) were purchased from Nacalai Tesque (Kyoto, Japan). Pyrene, SDS and Rhodamine B were purchased from Tokyo Chemical Industry (Tokyo, Japan). All chemicals were used without further purification. Ultra pure H_2O (resistivity: 18 MΩ cm) was used throughout all experiments.

2.2. Preparation of lipid membrane

Ceramide III, palmitic acid and cholesterol was mixed in the ratio of 2:1:1 (w/w%). Successively, the lipid mixture was melted on a slide glass at 120 °C under atmosphere pressure in a glass tube oven. After melted completely, the droplet of melt was covered by a cover glass, and then it was cooled down to room temperature. The obtained thin film of lipids was used as a model SC in further experiments. This optimum condition on the preparation of the model SC was determined previously [20]. The pyrene-labeled lipid membranes were prepared as follows: 2.1 mg of pyrene was added to 6.4 mg of lipid mixture, Ceramide III, cholesterol or palmitic acid, and the thin film of lipid mixture or each lipid was prepared according to the procedure described above.

2.3. SDS treatment

The sandwiched lipid membrane was got in touch with an aqueous 10 wt.% SDS solution, which was either unlabelled or labelled with rhodamine B, through the gap between glass plates (see Fig. 1a). The lipid membrane was observed by optical and fluorescence microscopes at appropriate time periods. After 1 day, the lipid membrane was rinsed with water thoroughly, and a piece of membrane was collected and characterized by Fourier transform-infrared (FT-IR) absorption spectra.

2.4. Characterization of lipid mixtures

Morphology observations of lipid membranes by optical and fluorescence microscopy were carried out using a TE-2000 U-EP-FL microscope (Nikon, Japan). FT-IR absorption spectra of lipids using KBr pellets were recorded on a Bio-Rad Digilab FTS-60A spectrometer in a transmittance mode at the range of 4000–400 cm^{-1} with 64 scans at 1 cm^{-1} resolution.

3. Results and discussion

The prepared membrane of lipid mixture was translucent (Fig. 1b) and indicated the existence of domains in the mixture under the optical microscope (Fig. 1c). This inhomogeneous texture suggests the micro phase separation of lipid mixture. After the membrane was exposed on a SDS solution, the inhomogeneous texture was emphasized to be a sponge structure up to the depth of 200 μm, and "myelin-form" [21] came up from the interface of membrane of lipid mixture (Fig. 2). This change suggests that the lipid mixture is phase-separated into two parts: one is rather stable on SDS treatment and remains sponge-like texture, and another is easily affected by the SDS treatment and eluted from the membrane to produce the myelin-form.

After 1 day of SDS treatment, the myelin-form and a SDS solution were removed and the sponge and bulk regions were separately collected for measuring FT-IR absorption spectra (Fig. 3). As evidenced by the comparison with a spectrum of each lipid, a spectrum of bulk membrane was the superposition of those of constituent

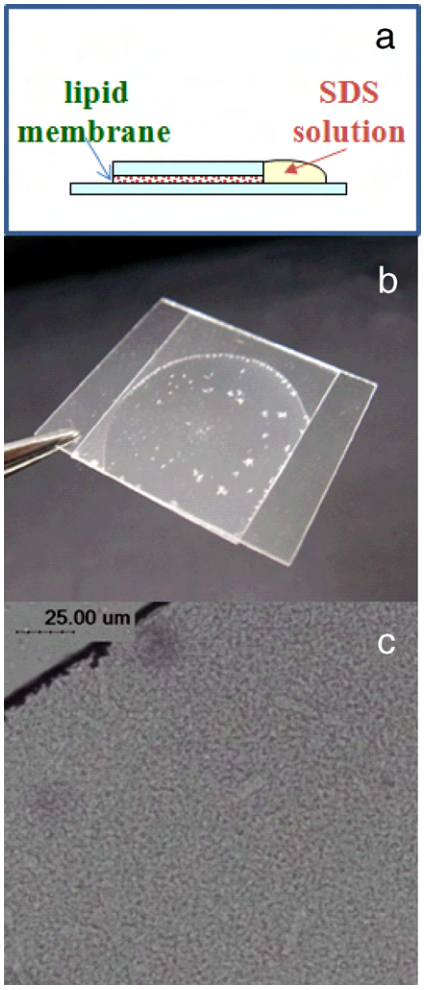

Fig. 1. A cartoon (a) and a photograph (b) of lipid membrane sandwiched between plates, and an optical microscopic image (c) of the membrane.

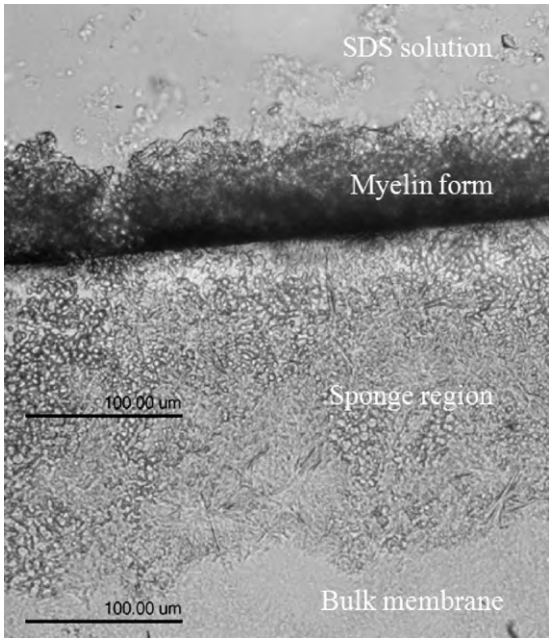

Fig. 2. An optical microscopic image of membrane of lipid mixture after exposed on an aqueous10% SDS solution.

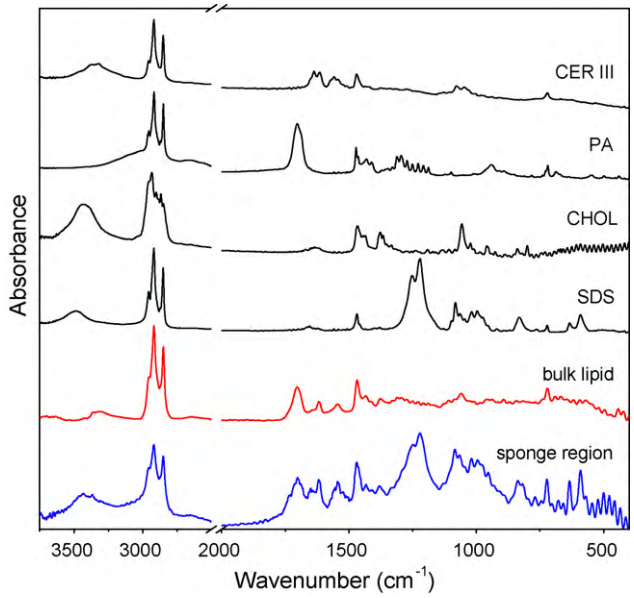

Fig. 3. Infrared absorption spectra of lipid membranes after exposed on an aqueous10% SDS solution in comparison with component lipids.

lipids. On the other hand, existence of SDS in the sponge structure was confirmed from a strong absorption band of sulphonate (1230 cm^{-1}), supporting that SDS molecules penetrate into the membrane of lipid mixture. Furthermore, the stretching mode of hydroxyl group (3300 cm^{-1}) in the bulk membrane shifted to high frequency (~3400 cm^{-1}) in the sponge structure. This change indicates that hydrogen bond in the sponge structure weakens and the packing of lipids is loosened. A relative intensity of an amide C=O (1650 cm^{-1}) band against a carboxylic acid (1720 cm^{-1}) band in sponge structure was higher than that in translucent (bulk) part. That is, relative concentration of ceramide III became higher than those of the other lipids in the sponge structure. It is suggested that the lipid mixture is varied in constituents to be ceramide-rich after SDS treatment.

Using fluorescent probes, the elution behaviour of lipids was elucidated by microscopic observation (Fig. 4). When the probe (rhodamine B) was in the aqueous phase, a dark area on a fluorescent micrograph spread into wider region than the myelin-form which extended from the interface of membrane of lipid mixture and determined on an optical micrograph (Fig. 4a). This difference on the elution border between observation on optical and fluorescence microscopy indicates the existence of at least two lipids eluted. The border between sponge region and bulk membrane occurred at almost same depth of the membrane in optical and fluorescence microscopic images, indicating the penetration of rhodamine B with SDS into the membrane. Meanwhile the fluorescence of lipophilic probe (pyrene) was localized at bulk membrane but it was much weaker in the sponge region than in the bulk membrane and even in the myelin-form (Fig. 4b). This suggests that the significant fraction of pyrene is eluted from the membrane of lipid mixture together with the elution of lipids.

To identify the lipids eluted from the membrane of lipid mixture, a film of each component lipid mixed with pyrene was separately prepared and exposed on a SDS solution. The observations on optical and fluorescence microscopes were carried out before and after treatment with an SDS solution (Fig. 5). The microscopic images indicated that the ceramide membrane was not affected by the SDS treatment. Meanwhile the texture of the membrane of palmitic acid seemed to be less affected after the SDS treatment, because there was no myelin-form on an optical microscopic photograph. However, the difference was observed on fluorescence microscopic images: The border of fluorescence of pyrene came down inside of membrane during SDS treatment. These results indicate that palmitic acid elutes into the SDS solution without remarkable texture like myelin-form. Significant variation caused by SDS treatment was found in every photographs of cholesterol. Cholesterol was eluted in the SDS solution and generated the myelin-form as seen in an optical microscopic observation. In the fluorescence microscopic images, the border of fluorescence by pyrene probe came down with SDS treatment as with a case of palmitic acid. This implies that cholesterol elutes easily in a SDS solution and generates myelin-form.

From these results, the elution behaviour from the membrane of lipid mixture can be elucidated as below: (1) the membrane of lipid mixture separates into ceramide-rich and ceramide-poor domains by the micro phase separation; (2) after SDS treatment, ceramide-rich phase remains as sponge texture; and (3) from the ceramide-poor domain, cholesterol elutes in a SDS solution with producing myelin-form, and palmitic acid also elutes but is not generated myelin-form.

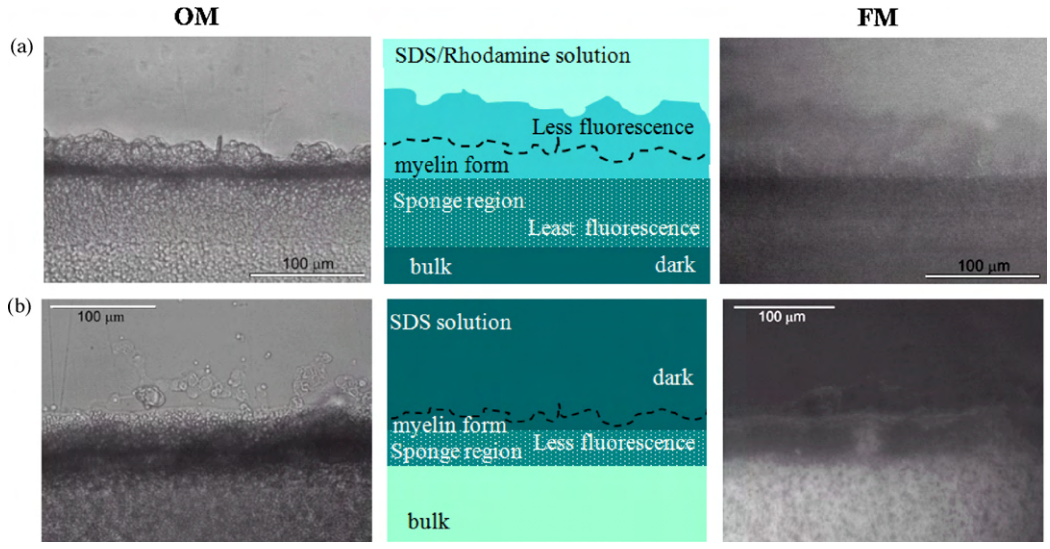

Fig. 4. Optical and fluorescence microscopic images of lipid membranes after exposed on an aqueous10% SDS solution. (a) Rhodamine was solved in an SDS solution; (b) pyrene was mixed in lipid membrane.

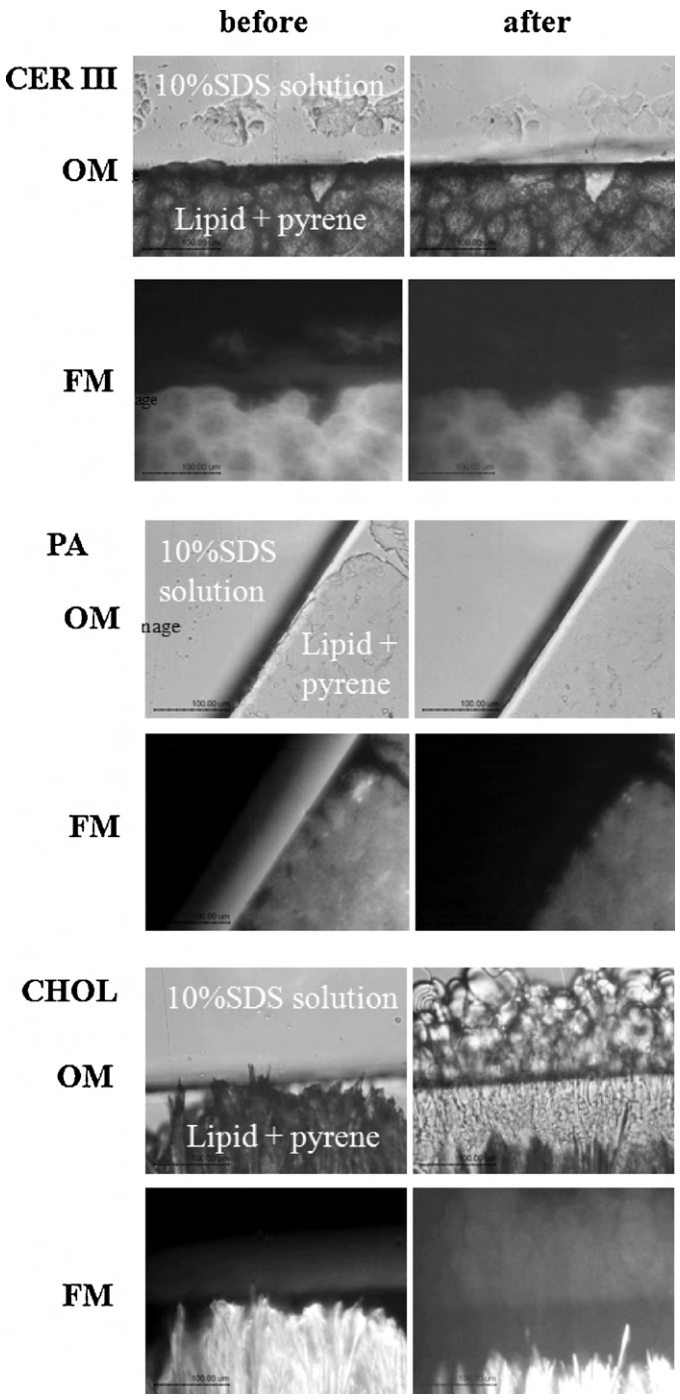

Fig. 5. Optical and fluorescence microscopic images of pyrene-doped component lipids after exposed on an aqueous10% SDS solution.

4. Conclusions

When human skin is exposed to external stimulus, it is common knowledge that there is damage on the skin. One of external stimuli is a detergent which is used for washing human body, clothes and dishes, but the mechanism and process of damage from chemical aspect have not necessarily been elucidated. Therefore, the challenge in the present work was to clarify the phenomenon on the human skin which was made contact with the detergent. The membrane of lipid mixture consisting of ceramide, palmitic acid, and cholesterol was prepared as mimicry of human SC and exposed on an aqueous solution of SDS, one of typical surfactants. It became evident from microscopic observation that the elution of components from mimetic lipid membrane is selectively performed, that is, cholesterol is easy eluted and palmitic acid is also eluted, different from the remanence of ceramide. Especially, the appearance of myelin-form during the elution of cholesterol can be useful as a conventional marker on the elution of cholesterol. On the other hand, the fluorescence-probe method makes us to estimate the total elution of lipids from the bulk.

The phenomenon of elution of lipids may be related to the phase separation of lipid mixture into ceramide-rich and ceramide-poor domains and the selective swelling of ceramide-poor domain by hydration of lipid mixture, following the penetration of surfactant which is an indispensable process because the elution of lipids by surfactant proceeds. The latter phenomenon should occur especially for cholesterol and involve its elution as myelin-form. The swelling and elution behaviours of lipids could throw light on the interpretation of damaging process on human SC caused by surfactants.

The mechanism and process of damage by SDS on SC have been investigated using a model membrane, the process has been elucidated, and the mechanism has been proposed [22]. Then the investigation was performed using mainly thermal, spectroscopic and diffraction methodologies. Although the results obtained in the present work are alike as those from previous report, the present investigation was carried out mostly by means of optical and fluorescence microscopic techniques which are convenient and concise. Therefore, the present work indicates the simple and easy sensing system for testing the effect of external stimulation.

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