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In vitro Analysis of the Effect of Alkyl-Chain Length of Anionic Surfactants on the Skin by Using a Reconstructed Human Epidermal Model

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Abstract: We investigated the effect of the alkyl-chain length of anionic surfactants on the skin using an *in vitro* model. The evaluated anionic surfactants were sodium alkyl sulfate (AS) and sodium fatty acid methyl ester sulfonate (MES), which had different alkyl-chain lengths (C8–C14). Skin tissue damage and permeability were examined using a reconstructed human epidermal model, LabCyte EPI-MODEL24. Skin tissue damage was examined by measuring cytotoxicity with an MTT assay. Liquid chromatography/tandem mass spectrometry (LC/MS-MS) and liquid chromatography/mass spectrometry (LC/MS) were used to detect surfactants that permeated into the assay medium through an epidermal model. To assess the permeation mechanism and cell damage caused by the surfactants through the epidermis, we evaluated the structural changes of Bovine Serum Albumin (BSA), used as a simple model protein, and the fluidity of 1,2-dipalmitoyl-sn-glycero-3-phosphpcholine (DPPC) liposome, which serves as one of the most abundant phospholipid models of living cell membranes in the epidermis. The effects of the surfactants on the proteins were measured using Circular Dichroism (CD) spectroscopy, while the effects on membrane fluidity were investigated by electron spin resonance (ESR) spectroscopy. ET50 (the 50% median effective time) increased as follows: C10 < C12 < C8 < C14 in AS and C8, C10 < C12 < C14 in MES. The order of permeation through the LabCyte EPI-MODEL24 was C10 > C12 > C14, for both AS and MES. For both AS and MES, the order parameter, which is the criteria for the microscopic viscosity of lipid bilayers, increased as follows: C10 < C12 < C14, which means the membrane fluidity is C10 > C12 > C14. It was determined that the difference in skin tissue damage in the LabCyte EPI-MODEL24 with C10 to C14 AS and MES was caused by the difference in permeation and cell membrane fluidity through the lipid bilayer path in the epidermis.

Key words: reconstructed human skin, LabCyte EPI-MODEL24, anionic surfactant, MTT, permeability, Electron Spin Resonance (ESR)

1 INTRODUCTION

Because detergents are frequently used in household cleaners, cosmetic products, and other materials that come into contact with skin, there have been numerous studies evaluating the effects of detergents on skin[1–6]. Most of these skin irritation tests have been performed in guinea pigs or using closed patches of human skin. The irritation test scores were reported to reach a maximum at the alkyl-chain length of C10, C12[6]. From these results, it was suggested that the chain length of the hydrophobic group of AS is an important factor in skin irritation.

Recently, with the ongoing considerations for animal welfare and the enforcement of a new cosmetics law (EU Directive 2003/15/EC, 2003) and REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) legislation in Europe, research efforts have focused on finding alternatives to *in vivo* animal testing of skin irritation. As part of this effort, an *in vitro* method for testing skin irritation/corrosivity using a reconstructed human epidermal model was evaluated and accepted by ECVAM (Eu-

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ropean Center for the Validation of Alternative Methods), ICCVAM (The Interagency Coordinating Committee on the Validation of Alternative Methods), and OECD (Organisation for Economic Co-operation and Development). Three validated *in vitro* test methods were adopted as OECD Test Guideline (TG) 439 in 2010. LabCyte EPI-MODEL24 is a commercially available *in vitro* human epidermal model that was assessed according to the Performance Standards and then added to TG 439 in 2013[7].

For the present study, we selected two anionic surfactants, AS and methyl ester sulfonate (MES), which are commonly used in personal care and household products. MES, in particular, has a sulfonyl group in the alpha position and has attracted considerable attention as a sustainable material that can be obtained from plants, such as palm oil. Therefore, many studies have been performed to assess the physicochemical properties[8-12], applicability in heavy-duty surfactants[13, 14], and environmental toxicity[15, 16] of MES. Here, we evaluated the effects of the alkyl-chain length of the anionic surfactants, AS and MES, on cytotoxicity and epidermal permeability using a LabCyte EPI-MODEL24. In addition, we compared the effect of alkyl-chain length of C8, C10, C12, and C14 for AS and MES on cell membrane fluidity, which plays an important role in cytotoxicity as related to skin tissue damage and lipid pathway permeability.

2 EXPERIMENTAL

2.1 Materials

The molecular structures of AS and MES are shown in Table 1. Sodium octyl sulfate (C8AS-Na, 90% purity) and sodium decyl sulfate (C10AS-Na, 90% purity) were purchased from Kanto Chemical, Inc. (Tokyo, Japan). Sodium dodecyl sulfate (SDS; C12AS-Na, 95% purity) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Sodium tetradecyl sulfate (C14AS-Na, 99% purity) was purchased from Nikko Chemical Co., Ltd. (Tokyo, Japan). Sodium decanoic acid methyl ester sulfonate (C10MES-Na, 100% purity), sodium dodecanoic acid methylester sulfonate (C12MES-Na, 100% purity), sodium tetradecanoic acid methylester sulfonate (C14MES-Na, 93.8% purity) and sodium hexadecanoic acid methylester sulfonate (C16MES-Na, 100% purity) were synthesized at Lion Corporation (Tokyo, Japan). All test samples were diluted with distilled water (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan) to 55 mM aqueous solution.

Table 1 Chemical structures of AS and MES.

Surfactant	Chemical structure	Alkyl length (R)	R1
C8 AS	R_1-O-SO_3Na	C8	$-C_8H_{17}$
C10AS		C10	$-C_{10}H_{21}$
C12AS		C12	$-C_{12}H_{25}$
C14AS		C14	$-C_{14}H_{29}$
C10MES	$R_1-CH(SO_3Na)-COOCH_3$	C8	$-C_8H_{17}$
C12MES		C10	$-C_{10}H_{21}$
C14MES		C12	$-C_{12}H_{25}$
C16MES		C14	$-C_{14}H_{29}$

MTT (3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, 98% purity) and BSA (bovine serum albumin, 93.9% purity) were purchased from Sigma-Aldrich Japan K.K. (Tokyo, Japan). DPPC (dipalmitoylphosphatidylcholine, 100% purity) and the LabCyte EPI-MODEL24 were purchased from the NOF Corporation (Tokyo, Japan) and Japan Tissue Engineering Co., Ltd. (Aichi, Japan), respectively.

2.2 Testing for skin tissue damage with the LabCyte EPI-MODEL24

LabCyte EPI-MODEL24 tissues were aseptically removed from the transport agarose medium, transferred into 24-well plates (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) containing the assay medium (500 μL), and pre-incubated for 1 h in a 5% CO_2 humidified atmosphere at 37℃. After pre-incubation, 55 mM aqueous solution of each test sample (50 μL) was applied to the stratum corneum side of the LabCyte EPI-MODEL24 tissue in a 5% CO_2 humidified atmosphere at 37℃. All test samples were tested three times under the same conditions. In addition, three tissues serving as negative controls were treated with 50 μL distilled water, and those treated with C12AS (SDS) were considered the positive controls. After 0.25, 0.5, 1.5, 3, 4.5, and 6 h of exposure, respectively, each tissue sample was carefully rinsed with 800 μL phosphate-buffered saline (PBS(-)) 3 times using a micropipette to remove any remaining test chemicals from the surface. The blotted tissues were then transferred to new wells of 24-well plates containing 500 μL/well of MTT medium (1 mg/mL) for the MTT assay. The tissues were then incubated for 3 h in a 5% CO_2 humidified atmosphere at 37℃.

Tissue discs were cut off from the insert wells and then transferred to microtubes containing 200 μL of isopropanol. Formazan extraction was performed for 48 h at 4℃ in the dark, following the manufacturer's recommendations. Subsequently, 150 μL of the extracts was transferred to a 96-well plate. The optical density (OD) was measured at 570 nm using a spectrophotometer (Multiskan JX; Thermo Fisher Scientific, Kanagawa, Japan). Isopropanol was used as a blank. Viability was expressed as a percentage (%) of the mean negative control optical density (OD) and was calculated as follows:

[(OD treated − OD blank)/(OD negative control − OD blank)] × 100

The mean of the three values from identically treated tissues was plotted, and the ET50 (50% median effective time) was then calculated from the approximation curve. All experiments were performed three times independently[17], and the mean value of the ET50 was shown as skin tissue damage.

2.3 Determination of surfactant permeability with the LabCyte EPI-MODEL24

Detection of the surfactant penetrating into the tissue was affected by impurities in the medium. Therefore, after pre-incubation, the medium was removed to eliminate the effects of these impurities, and the tissues were incubated with PBS(-) (500 μL).

A 50 μL aliquot of aqueous solution containing 55 mM of each test sample was applied to the stratum corneum side of a LabCyte EPI-MODEL24 tissue for 0.25–4.5 h at 37℃ in a 5% CO_2 humidified atmosphere. Each test sample was assessed three times under the same conditions. Each test sample was removed with a pipette and washed with 800 μL of PBS(-) four times. The permeated sample PBS(-) (500 μL) was collected and mixed with acetonitrile (500 μL). The amount of surfactant in PBS(-) was measured by liquid chromatography/tandem mass spectrometry (LC/MS-MS) and liquid chromatography/ mass spectrometry (LC/MS).

LC/MS-MS analysis was performed using an Alliance 2695 system (Nihon Waters K.K., Tokyo, Japan) hyphenated with a Quattro Micro API equipped with an electrospray ionization (ESI) source. The separation was achieved using a reversed-phase Pegasil-ODS 2.1 mm I.D. × 150 mm L column (5 μm) at 40℃. The following solvents were used: 10 mM ammonium acetate in water (A) and 10 mM ammonium acetate in acetonitrile/methanol/water (90:5:5) (B). The multiple reaction monitoring (MRM) mode was used, and the specific parent/daughter transitions were 293.13/79.96 (C10MES), 321.19/79.96 (C12MES), and 349.20/79.96 (C14MES).

LC/MS analysis was performed using a Prominence (Shimadzu, Kyoto, Japan) system hyphenated to a Quattro Micro API equipped with an ESI source. The separation was achieved using a reversed-phase Pegasil-ODS 2.1 mm I.D. × 150 mm L column (5 μm) at 40℃. The mobile phase was 10 mM ammonium acetate in acetonitrile/water (70:30). The selected ion monitoring (SIM) mode was used along with the following positive ion ESI source conditions: m/z 265.10, 293.10, 321.10, and 349.15, for C8, C10, C12, and C14 in MES, respectively, and m/z 209.10, 237.10, 265.15, and 293.15, for C8, C10, C12, and C14 in AS, respectively. All LC/MS-MS and LC/MS data were processed by MassLynx version 4.0 NT Quattro data acquisition software (Shimadzu, Kyoto, Japan).

The Flux (mass/area-time) was determined from the slope of the plot of cumulative detergent molecules per unit area in the receptor solution over time (nmol/cm2/h). Time points before surfactant detection in the receptor solution were not used in the determination of the slope.

2.4 Evaluation of structural changes in a protein model

BSA was used as the simplest protein model for which the denaturation of its structure is reportedly related to skin damage[18]. Structural changes in BSA due to the interaction of AS and MES were examined using circular dichroism (CD) spectroscopy. It is known that the α-helical structure of BSA shows a strong negative double minimum at 207 and 222 nm, and the negative ellipticity of BSA solution is decreased when the α-helical structure is destroyed. The molar ellipticity, $[\theta]_{222}$, of the mixed PBS(-) (50 mM, pH 7) solution containing 1.42×10^{-6} M BSA and 55 mM surfactants at 222 nm were measured by a J-500 CD spectrophotometer (JASCO Corporation, Tokyo, Japan) at 25℃.

2.5 Evaluation of fluidity using a cell membrane model

DPPC is one of the most abundant phospholipid models of the living cell membrane in the epidermis[19]. Multi-lamellar vesicles (MLV) of DPPC containing 5-doxylstearic acid (5NS) were prepared according to methods described as follows. A thin lipid film was formed on the surface of the flask by removing the solvent from the chloroform solution containing DPPC and 5NS. After adding PBS(-) (50 mM, pH 7) to the film, the mixture was heated to 70℃ and stirred by vortexing for 10 minutes. The size of the MLV in the suspension ranged from 2–3 μm. After the MLV dispersion and surfactant solutions were added and mixed, the combined solution was immediately infused into a cell for liquid samples. Ten minutes later, it was analyzed using a JES-FA100 electron spin resonance (ESR) spectrophotometer (JEOL Ltd., Tokyo Japan) in the magnetic field range of 330 ± 5 mT at 37℃. The concentrations of DPPC, 5NS, and surfactant in the mixed solution were 7 mM, 1×10^{-4}, and 5 mM, respectively. Figure 1 shows the typical spectrum of 5NS in the lipid bilayer. The order parameter, which is the value representing the index of the microscopic viscosity of the spin probe, was calculated using the following equation with the value of 2A//and $2A\perp$[20]:

$$S = 2(A// - A\perp)(A_{xx} + A_{yy} + A_{zz})/(2A_{zz} - A_{xx} - A_{yy})(A// + 2A\perp) \quad (1)$$

$(A_{xx}, A_{yy}, A_{zz} = 0.63, 0.58, 3.36\ \mathrm{mT})$

2.6 Binding behavior of surfactants to the lipid bilayer

The binding behavior of surfactants to the lipid bilayer was assessed by MicroCal VP-Isothermal Titration Calorimetry at 37℃. The test samples (50 mM PBS(-), pH 7) were titrated into an MLV dispersion (0.1 mM DPPC) as de-

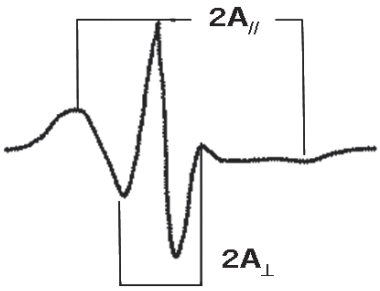

Fig. 1 ESR spectrum of 5NS in the lipid bilayer of DPPC.

scribed above in section 2.5.

3 RESULTS

3.1 Evaluation of epidermal damage using the LabCyte EPI-MODEL24

As shown in Fig. 2, the ET50 values for alkyl-chain lengths C8, C10, C12, and C14 in AS were found to be 2.1 h, 0.5 h, 0.8 h, and 2.5 h, respectively. The maximum degree of skin tissue damage by AS was observed at the alkyl-chain length of C10. On the other hand, the ET50 values for alkyl-chain lengths C8, C10, C12, and C14 in MES were found to be 0.7 h, 0.7 h, 1.5 h, and 4.4 h, respectively. The maximum degree of skin tissue damage by MES was observed at the alkyl-chain lengths of C8 and C10.

3.2 Surfactant permeability assay using the LabCyte EPI-MODEL24

The Flux (moles/area-time) was determined from the slope of the plot of cumulative surfactant moles per unit area in the assay medium (Table 2). The permeability rates (Flux) of AS and MES through the LabCyte EPI-MODEL24 tissues were extremely high at a carbon-chain length of C8 and decreased with alkyl chain elongation.

Table 2 Permeation rate (Flux) of AS and MES in the LabCyte EPI-MODEL24.

Alkyl Chain length (R)	Flux (nmol/cm^{2}h)	
	AS	MES
C8	939.03	843.70
C10	495.08	70.45
C12	32.86	27.69
C14	1.61	5.82

3.3 Effect of surfactants on substances in the epidermis

The LabCyte EPI-MODEL24 possesses the stratum corneum structure in the upper site of the epidermis just found in the native human epidermis. While it is has been shown that the stratum corneum structure in the LabCyte EPI-MODEL24 delays the permeation of chemicals[21], the barrier function of the LabCyte EPI-MODEL24 is weaker than that observed in humans and animals[22]. Surfactants, such as AS and MES, must easily permeate and disturb the protein and lipid domains in the epidermis.

The structural changes in BSA and in the MLV of DPPC were used as simple models of substances in the epidermis, such as the cell membrane of keratinocytes and stratum corneum, and were examined after surfactants were added.

Figure 3 shows the change in molar ellipticity, $[\theta]_{222nm}$, due to the α-helix structure of BSA by mixing with AS and MES. The $-[\theta]_{222nm}$ of BSA was 11×10^6. After adding AS and MES on each alkyl-chain length from C10 to C14, all of the $-[\theta]_{222nm}$ values decreased around 9×10^6, independent of the alkyl-chain length. In contrast, the $[\theta]_{222nm}$ of BSA was not influenced by adding AS and MES with the alkyl-chain length of C8.

Figure 4 shows the fluidity of the bilayer when AS and MES were added to the MLV dispersion of DPPC containing 5NS as a spin probe. The fluidity of the lipid bilayer

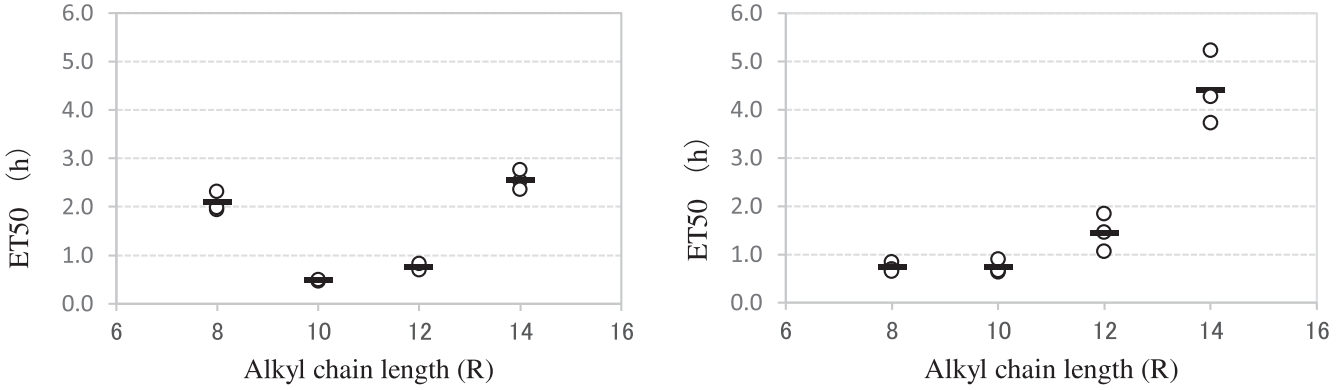

Fig. 2 Skin tissue damage of AS and MES with LabCyte EPI-MODEL24. LabCyte EPI-MODEL24 was treated with C8-14 AS (a) or MES (b) for 0.25-6 h at 37℃. Cell viability was measured by the MTT assay, and ET50 was calculated. The open circle represents each ET50 values, and the line represents mean values.

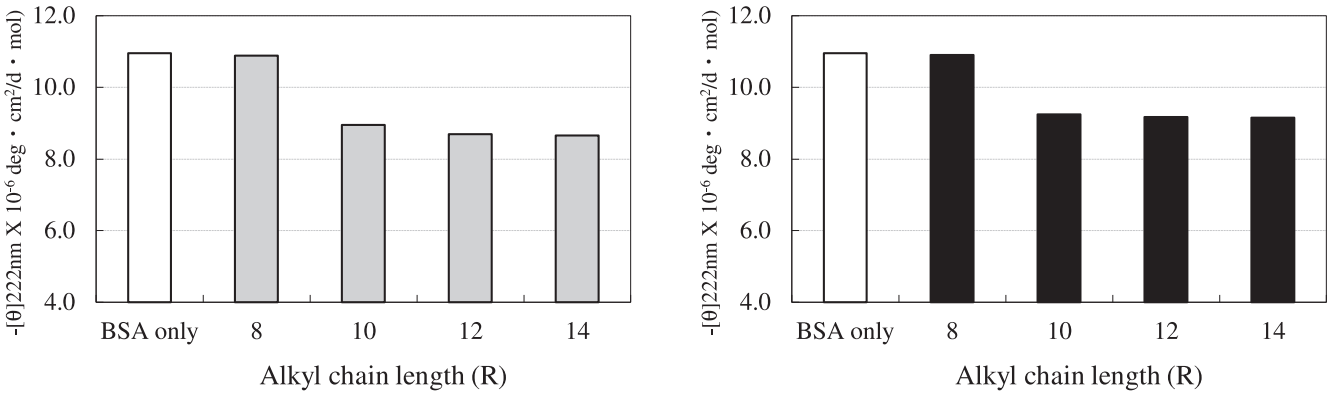

Fig. 3 Molar ellipticity of BSA (50 mM PBS (-), pH 7) at 222 nm, $[\theta]_{222}$, by mixing AS (a) and MES (b).

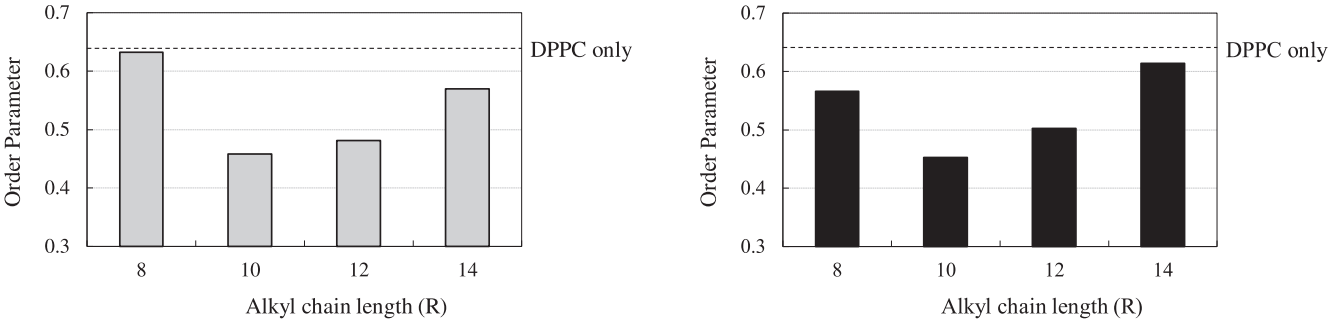

Fig. 4 Order parameters of the lipid bilayer after mixing AS (a) and MES (b) into the MLV dispersion.

showed by order parameters decreased significantly with alkyl-chain lengths of C10 and C12, and decreased slightly with alkyl-chain lengths of C8 and C14. A strong correlation between the alkyl-chain length and the order parameters was observed for AS and MES with an alkyl-chain length over C10, in the following order: C10＜C12＜C14.

3.4 Binding behavior of surfactants to the lipid bilayer

When the surfactant molecules adsorbed to the lipid bilayer at a low concentration, the lipid bilayer was disturbed by monomer binding and formed mixed micelle-like domains consisting of lipid and surfactant molecules. At a high surfactant concentration, solubilization of lipid molecules occurred, and the amount of adsorbed surfactant molecules increased[23]. Figure 5 shows the binding enthalpy curves resulting from titration with AS or MES into the MLV dispersion of DPPC MLV. An endothermic enthalpy was observed in the region with a low surfactant titration concentration. This finding revealed that the surfactant monomer bound to the lipid bilayer through hydrophobic interactions with the alkyl chains. Although the endothermic enthalpy of longer alkyl-chain lengths increased, the binding ratio decreased. The binding parameters, the binding constant (K) and the binding ratio (N), were larger for AS than for MES, indicating that AS had greater binding ability than MES. On the other hand, AS and MES molecules with an alkyl-chain length of C8 were hardly able to bind to the lipid bilayer and showed only slight binding at a high surfactant concentration.

4 DISCUSSION

In vivo evaluation of skin irritation that may be caused by raw materials and products that are applied to skin has generally been performed using the Draize Test with animal skin or the patch test with human skin. In this study, we investigated the potential epidermal damage of two anion surfactants without using any animals the "3Rs" in animal research, reduction, replacement, and refinement.

An established guideline (OECD TG 439) exists for a skin irritation test method using a reconstructed human epidermal model. This test method (OECD TG 439) is effective for the identification of hazards in chemical irritants; however, the strength of irritation cannot be evaluated with this method. In this study, we were able to successfully compare the tissue damage grade of anionic surfactants by modifying the test method.

ET50 values were used in our evaluation as indicators of skin tissue damage, and damage caused by AS was highest at alkyl-chain lengths of C10 and C12 (Fig. 2a). *In vivo* data from the primary skin irritation test in guinea pigs[6] and human patch test[24] previously showed that the skin ir-

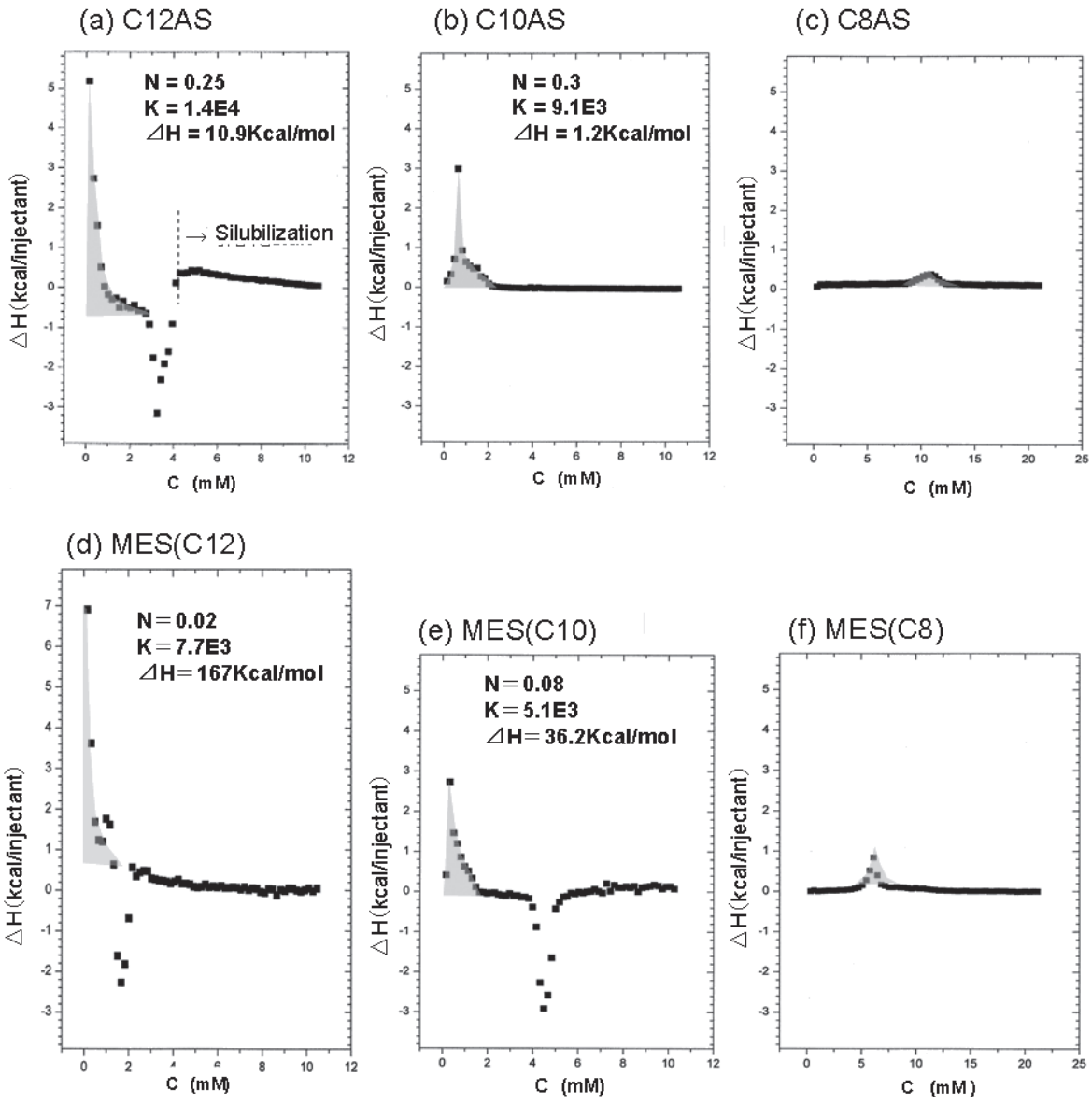

Fig. 5 Curve of the binding enthalpy of AS and MES to multi-lamellar vesicles of DPPC.

ritation score for AS was highest at alkyl-chain lengths of C10 and C12. Skin tissue damage caused by MES was highest at the alkyl-chain lengths of C8 and C10, which was shorter than that of AS (Fig. 2b) and consistent with data from primary skin irritation tests in guinea pigs (unpublished data). The relationships between skin irritation and alkyl-chain length correlated to the epidermal damage data obtained in this study, suggesting that our method using LabCyte EPI-MODEL24 is useful for comparison of skin irritation caused by anionic surfactants.

The alkyl-chain length causing the strongest tissue damage was different for AS and MES, and this difference was likely due to the structural difference of the effective alkyl-chain length in AS and MES. Fujiwara *et al*. reported that the cmc of MES was lower than that of AS[10]. Since the methylester group and the methine group in the MES head group contribute to hydrophobicity, the effective alkyl-chain length of MES is one or two carbon atoms larger than that of AS when they have the same alkyl-chain length, R. Thus, this is the reason why the skin tissue damage of MES with alkyl-chain length of C8 was similar to that of AS with the alkyl-chain length C10.

So far, the degree of skin tissue damage has been discussed with the permeability and the cytotoxicity in the epidermis. Based on our results, the Flux for both AS and MES with C10 to C14 correlated with their ET50 values (Figs. 6a and 6b), indicating that the skin tissue damage is greater when the surfactant easily permeates.

In the epidermis, there is the stratum corneum layer and the living cell layer, which includes a granular layer, spinos

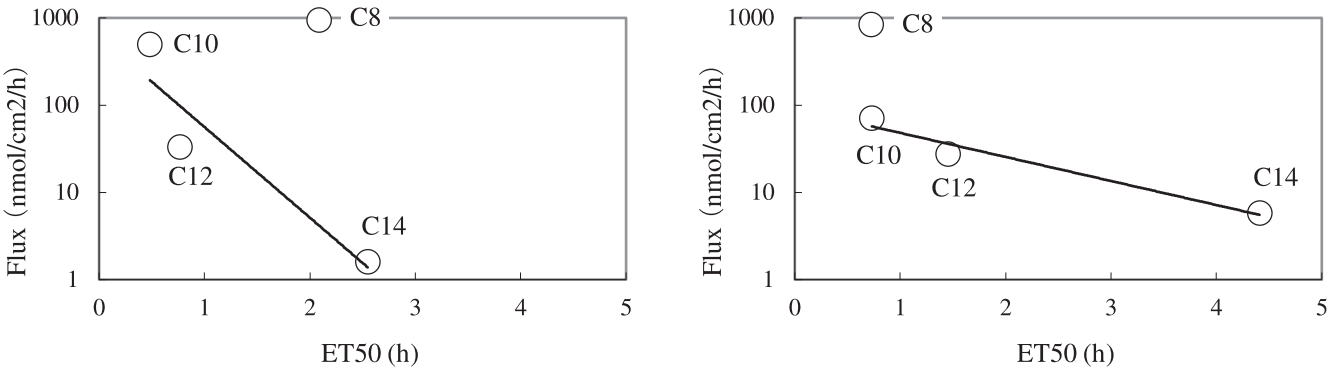

Fig. 6 Correlation between the permeation rate (Flux) and skin tissue damage (ET50) with LabCyte EPI-MODEL24. The open circle indicates each data of AS (a) and MES (b). Correlations between Flux and ET50 are shown by the solid line in C10-14.

layer, and basal layer. The stratum corneum is constructed of hardened proteins (keratins), desmosomes, and lamellar intercellular lipids. The living cell layer consists of the membrane proteins and the lipid bilayer. It is known that the interaction between the surfactant and living cells leads to a disturbance in the cell structure, promoting permeation and inducing skin irritation[25].

The structural changes observed in BSA serve as the simplest model of proteins, and it was shown that these structural changes were independent of the alkyl-chain length (Fig. 3) when the permeability was greater for AS and MES, with decreasing alkyl-chain length from C14 to C10. It is considered that all of the surfactants have enough influence on the protein structural changes under our experimental conditions.

In contrast, the fluidity of DPPC MLV as a lipid bilayer model, was greater with decreasing alkyl-chain length (Fig. 4), when the permeability was greater for AS and MES with C10 to C14. It is generally known that many hydrophobic penetrants induce the fluidization of the lipid bilayer in the epidermis[26]. The stratum corneum layer in the epidermis is rich in ceramide, cholesterol, and free fatty acids[27], and the cell membrane of the living cell layer is rich in phospholipids, triglycerides, and cholesterol. Luiz *et al.*[28] reported that the fluidization of DPPC by 1, 8-cineole, which can interact with the hydrophobic part of lipids, is comparable to that of the stratum corneum in terms of ESR measurement. DPPC MLV was used as a lipid bilayer model of the epidermis in our study. Although there are limitations, it has been suggested that fluidization caused by surfactants in the epidermis model is roughly expected with the DPPC model membrane since the living cell contains phospholipids[19].

On the other hand, fluidization of the lipid bilayer is related to cell damage. Membrane fluidization was shown to be caused by the binding of monomer surfactant to the lipid bilayer at low surfactant concentration under cmc[29]. In addition, it has been shown that carboxyfluorescein (CF) leakage from the internal water phase of liposomes, which is one measure for cytotoxicity[30], increases with enhanced membrane fluidity by binding of surfactant monomers[31]. This means that when the membrane is disturbed by substances, the cells incur damage leading to death.

Figures 7a, 7b, 8a, and 8b show the relationships of the order parameter with the Flux and the ET50, respectively. In AS and MES of C10 to C14, the permeability and the cell damage in the LabCyte EPI-MODEL24 were significantly correlated with the fluidity of the lipid bilayer. As the binding ratio was decreased with longer alkyl-chain length (Fig. 5), it is clear that the elongation in the alkyl chain led to decreased disturbance of the lipid layer, lower permeability, and less cell damage. Howes[2] reported the permeation coefficients of soap through isolated human epidermis. The permeability of soap of C12 to C16 chains decreased with an increase in the alkyl-chain length, and the maximum skin irritation of soap was observed at C12[25]. These results were similar to our results for AS and MES. Although additional studies are needed, the evaluation using the LabCyte EPI-MODEL is useful for understanding the effect of surfactant structure on permeability and cell damage.

In contrast, C8AS and C8MES were out of line from the relationship between permeability and skin tissue damage (Figs. 6a and 6b), and were off the correlations between the order parameter and the permeability and/or the skin tissue damage (Figs. 7a, 7b, 8a, and 8b). Compared to longer alkyl chains, C8AS and C8MES had a higher Flux (Table 2) and lower influence on the fluidity of the lipid bilayer (Fig. 4). The cmc of C8AS and C8MES were larger than those of AS and MES having longer alkyl-chain lengths (Tables 3a and 3b). Moreover, since binding enthalpy of C8AS and C8MES onto the lipid bilayer was less (Figs. 5c and 5f), it is suggested that their interaction with the hydrophobic portion of the lipid bilayer was weak. Based on these findings, it was presumed that C8AS and C8MES could passively diffuse through the concentration gradient

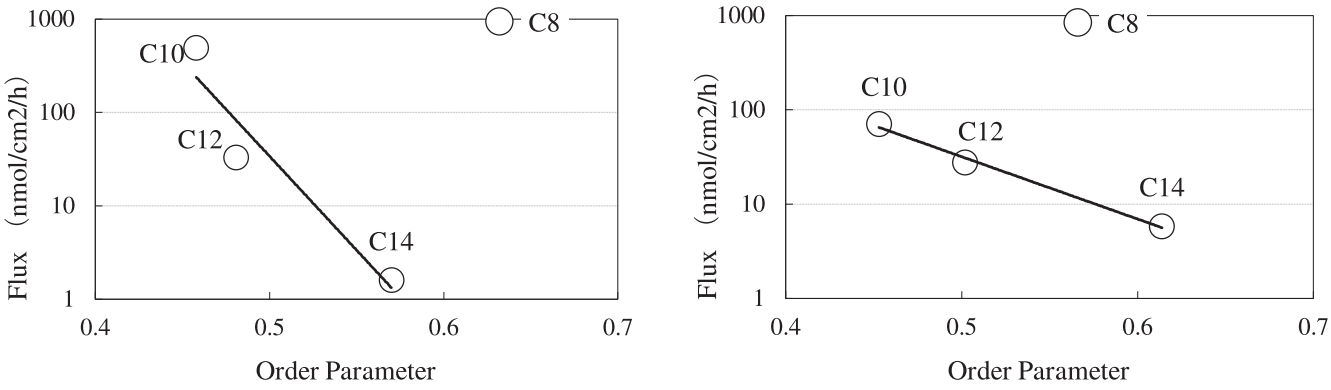

Fig. 7 Relationships between the fluidity of the lipid membrane (order parameter) and the permeation rate (Flux). The open ciecle indicates each data of AS (a) and MES (b). Correlation between order parameter and Flux are shown by solid line in C10-14.

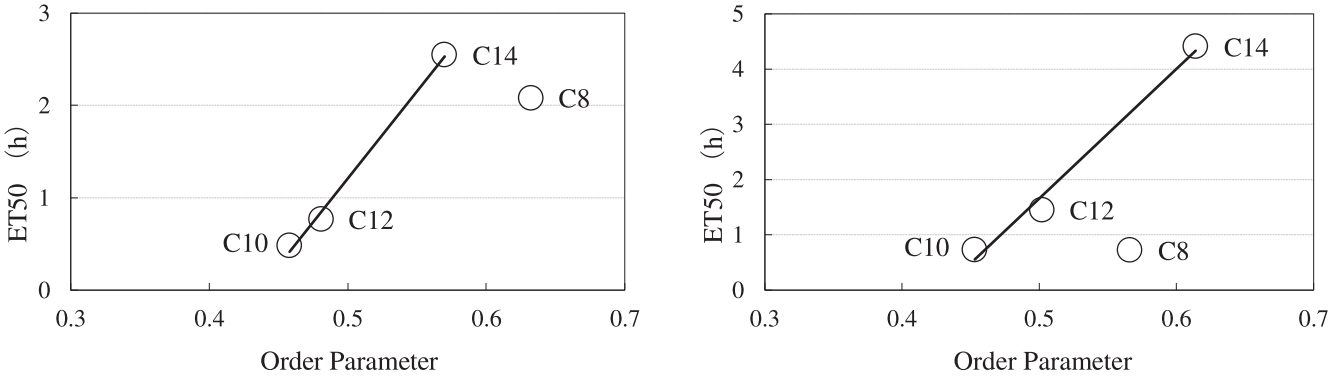

Fig. 8 Relationships between the fluidity of the lipid membrane (order parameter) and skin tissue damage (ET50). The open ciecle indicates each data of AS (a) and MES (b). Correlation between order parameter and ET50 are shown by solid line in C10-14.

Table 3 Critical micelle concentration (cmc) of AS and MES.

Alkyl Chain length (R)	cmc (mM/L)	
	AS	MES
C8	37	25
C10	16	2.7
C12	1.7	0.40
C14	0.18	0.037

in the epidermis. Therefore, since C8AS and C8MES could not accumulate in the cell membrane, it was expected that the accumulation of the lipid membrane on a keratinocyte or an organelle would be low. It was assumed that the lower binding ability of C8AS caused less epidermal damage, and this inference corresponded with previously reported results in which the toxicity of C8AS was remarkably lower than that of C12AS and C14AS using cultured HaCat cells[32].

On the other hand, C8 MES showed a high level of cell damage in spite of the lower binding enthalpy and the higher permeability than that of longer alkyl chain MES. Although we could not estimate the binding constant and binding ratio of C8MES, it is expected that C8MES has a lower binding constant and a higher binding ratio compared to C10 MES. The endothermic enthalpy of binding for C8MES means that the hydrophobic interaction was less. It appeared that more C8MES could not strongly bind, but rather, accumulated on the cell membrane because of the high flux in the epidermis, which then would cause C8MES damage to the cell membrane.

The epidermal cell damage caused by anionic surfactants, AS and MES, was evaluated using a reconstructed human epidermal model. The difference in the cell damage caused by the surfactants (C8, C10, C12, C14AS, and MES) was clarified by optimizing the application concentration and exposure time of samples. From our results, we concluded as follows: The skin tissue damage of AS and MES was highest at alkyl-chain lengths of C10 and C8, respectively. Skin tissue damage caused by MES at alkyl-chain lengths from C10 to C14 tended to be lower than that of AS with the same alkyl-chain length. The difference in the epidermal damage between AS and MES was likely due to dif-

ferences in permeability through the epidermal model and the cell membrane.

5 CONCLUSION

It was possible to compare the tissue damage grade of anionic surfactants using a reconstructed human epidermal model, LabCyte EPI-MODEL24. For AS and MES in the alkyl-chain lengths from C10 to C14, each increase in alkyl-chain length led to a decrease in skin tissue damage, as well as decreases in the Flux and the membrane fluidity of the lipid bilayer. These results demonstrate that permeation through the epidermal model and subsequent interaction with the cell membrane is one mechanism for epidermal damage caused by AS and MES.

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