J. Soc. Cosmet. Chem., **38**, 21–28 (January/February 1987)

Effects of surface active agents on stratum corneum cell cohesion

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Received August 20, 1986.

Synopsis

Stratum corneum was completely disrupted to intact individual corneocytes by immersion in a solution of sodium dodecylsulfate (SDS)/N,N-dimethyldodecylamine oxide (C_{12}DMAO) (2:8) mixture without mechanical or ultrasonic stimulation. On the other hand, stratum corneum immersed in solutions of Triton X-100, lipid solvents, keratolytic agents, or protein-denaturing agents was not split into any fragments, and individual corneocytes were few. Furthermore, the stratum corneum became less dispersable after methanol, ether, or chloroform/methanol (2:1) treatment. It was also found that the solution of SDS/C_{12} DMAO mixture markedly reduced the breaking strength of stratum corneum. The solution of SDS/C_{12}DMAO (2:8) mixture appears to dissolve the intercellular cement materials probably because of its low surface tension and electrostatic properties. This may induce the reduction of stratum corneum to individual corneocytes.

INTRODUCTION

The intercellular region of the stratum corneum and the adhesion between corneocytes are of great interest as they are closely related to desquamation and disease states such as ichthiosis. The combination of nonionic detergent, Triton X-100, and mechanical stimulation has been widely used to reduce stratum corneum to individual cells and to study the morphology of corneocytes or what components play important roles in cell-to-cell adhesion (1,2). Generally, mechanical stimulation is a necessary step and consists of scrubbing the skin surface vigorously with a rough implement *in vivo* or of grinding with a homogenizer *in vitro*. This technique, however, seems to force the stratum corneum to desquamate, and it would be impossible to completely solubilize the truly important components of cell-to-cell adhesion. Having investigated the effect of detergents on corneocyte adhesion, we found that stratum corneum was dissolved into intact individual cells only by immersion in aqueous solutions containing sodium dodecylsulfate and N,N-dimethyldodecylamine oxide without such mechanical stimulation.

The purpose of this study is to compare the effect of detergents with those of keratolytic

agents, protein-denaturing agents, and Triton X-100 on intercellular adhesion, judged by their ability to reduce stratum corneum to individual corneocytes.

MATERIALS AND METHODS

N,N-dimethyldodecylamine oxide (C_{12}DMAO), sodium dodecyl sulfate (SDS), Triton X-100, sodium laurate, polyoxyethylene(15) dodecyl ether, sodium dodecylbenzene sulfonate, dodecyltrimethyl ammonium chloride, N-dodecyl N,N-dimethylamino acetic acid, and salicylic acid (SA) were used for studying their effects on the adhesion of corneocytes. The above surfactants are commercially available. Stratum corneum was removed from guinea pig back by the heat-trypsinization method described by Kligman *et al* (3). The thickness of stratum corneum was examined with an electronic thickness gauge (Tokyo Seimitsu Co., Japan) at four different points.

Samples of stratum corneum measuring 10 × 10 mm were immersed in 10 milliliters of test solution and allowed to stand at 25°C for 1–30 days without mechanical stimulation. The degree of stratum corneum decomposition was observed directly with the unaided eye. Classification was made according to general patterns shown in Figure 1. The number of corneocytes dispersed in a test solution was counted using a Bürker-Türk haemocytometer and phase-contrast microscopy without staining. As the corneocyte count is believed to depend on the thickness of stratum corneum, the samples were selected to be as equal in thickness as possible. In addition to surfactants and protein-

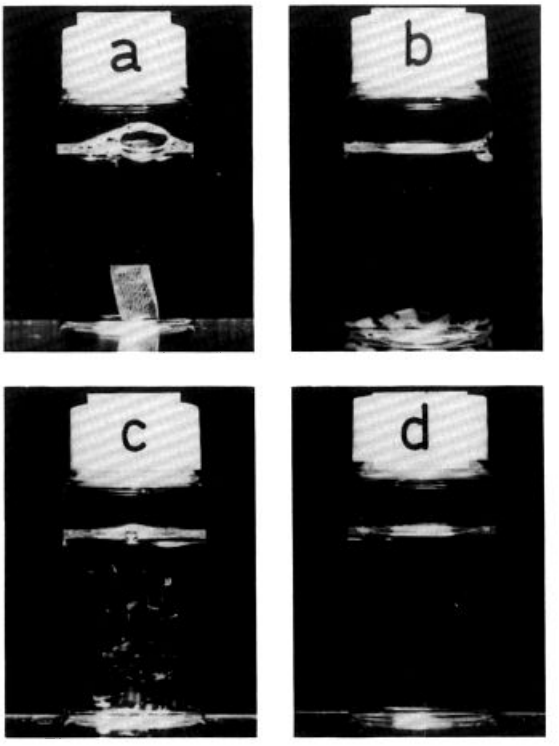

Figure 1. Classification of degree of decomposition of stratum corneum section (10 × 10 mm) as employed in Table I. (a) −; (b) +; (c) + +; (d) + + +.

denaturing agents (dimethyl sulfoxide and formic acid), the following keratolytic solutions were studied: saturated SA aqueous solution, 50% SA in ether (4), and a solution which contained 60% propylene glycol, 20% ethanol, 6% SA, and 14% distilled water (5).

The effects of lipid depletion by ether, ethanol, and chloroform/methanol (2:1) on the fragmentation of stratum corneum and on the counts of corneocytes released were examined. Stratum corneum decomposition and corneocyte counts were assessed by the same methods described above. The rheological properties of the stratum corneum (20 × 5 mm) were measured using an extensometer (non-commercial instrument) at a constant extension rate (3.14 mm/min) while immersed in a test solution at 25°C for 2 hrs. Two mechanical parameters, viz. modulus of elasticity (tensional force/strain; g/mm) and breaking strength (g), were obtained from the stress-strain curves. Each experiment was repeated four times.

RESULTS

The changes in stratum corneum sections immersed in test solutions (25°C) are presented in Table I. In most of the surfactant solutions including Triton X-100 and SDS, the stratum corneum was not split into fragments, though rolling or curling occurred. Even in keratolytic and protein-denaturing agents, the stratum corneum was not reduced to corneocytes. In sodium hydroxide solution the section of stratum corneum was split into many fragments. However, the fragments existed as large aggregates of corneocytes and could not be reduced further to individual intact corneocytes. On the other hand, the stratum corneum was gradually dispersed in C_{12}DMAO solution and finally disappeared into suspended individual corneocytes. It was comfirmed by microscopic observation that most of the stratum corneum cells in C_{12}DMAO solution retained their characteristic shape even after disruption to individual cells. The decomposition of stratum corneum was accelerated by addition of SDS to C_{12}DMAO solution. In a 0.1 M/l solution of SDS/C_{12}DMAO (2:8) mixture, the stratum corneum was completely reduced after 24 hrs immersion. However, the degree of stratum corneum decomposition was dependent on the concentration of the surfactant mixture, and it was not decomposed into fragments at less than the critical micelle concentration (ca. 5×10^{-4} M/l).

Changes in the corneocyte counts with time of stratum corneum immersion in typical surfactant and keratolytic solutions are shown in Figure 2. Corneocytes were rarely observed in Triton X-100, SDS, and saturated aqueous SA solutions even after immersion for 30 days. In solutions of C_{12}DMAO, the number of corneocytes gradually increased with increasing immersion time. Corneocytes were counted in high numbers in the SDS/C_{12}DMAO (2:8) mixture, and the count was almost constant with immersion time. These results corresponded to those from direct observation of the fragmentation of stratum corneum. However, in the case of sodium hydroxide solution there were almost no intact individual corneocytes in spite of the fragmentation of stratum corneum which occurred.

Figure 3 shows the relationship between the corneocytes counted in aqueous solutions of SDS/C_{12}DMAO mixtures (total concentration; 0.1 M/l) and the mole fraction of C_{12}DMAO. The corneocyte counts increased with increasing concentration of C_{12} DMAO and then decreased after reaching a maximum. The maximum occurred at a

Table I
Decomposition of Stratum Corneum Sections in Various Test Solutions

Test material	Concentration	1 Day	3 Days	8 Days	30 Days
Triton X-100	0.1 M/l	−	−	−	−
	0.1%	−	−	−	−
Polyoxyethylene(15) dodecyl ether	0.1 M/l	−	−	−	−
Sodium laurate	0.1 M/l	−	−	−	−
Sodium dodecylbenzene sulfonate	0.1 M/l	−	−	−	−
Dodecyltrimethyl ammonium chloride	0.1 M/l	−	−	−	−
N-dodecyl N,N-dimethylamino acetic acid	0.1 M/l	−	−	−	−
SDS	0.1 M/l	−	−	−	−
C_{12}DMAO	0.1 M/l	+	+ +	+ + +	+ + +
SDS/C_{12}DMAO (2:8)	0.1 M/l	+ + +	+ + +	+ + +	+ + +
	10^{-2} M/l	+ + +	+ + +	+ + +	+ + +
	10^{-3} M/l	+ + +	+ + +	+ + +	+ + +
	10^{-4} M/l	−	−	−	−
Saturated aqueous SA solution		−	−	−	−
50% SA in ether		−	−	−	
60% Propylene glycol, 6% SA, 20% ethanol, and 14% water		−	−	−	−
1 N NaOH		+	+ +	+ +	+ +
1 H HCl		−	−	−	−
Thioglycollic acid	1 M/l	−	−	−	−
Formic acid	90%	−	−	−	−
Dimethyl sulfoxide	1 M/l	−	−	−	−
Lactic acid	1 M/l	−	−	−	−
Ether		−	−	−	−
Ethanol		−	−	−	−
Chloroform/methanol (2:1)		−	−	−	−

−: no change. +: Stratum corneum was decomposed to a few fragments. + +: Stratum corneum was decomposed to many fragments. + + +: Stratum corneum disappeared, being decomposed to individual corneocytes.

mole fraction of 0.8 for C_{12}DMAO. In the case of immersion at 50°C, the corneocyte counts were increased more than at 25°C at every mole fraction of C_{12}DMAO, especially at 1.0.

The immersion in lipid solvent had no effect on the fragmentation of stratum corneum as shown in Table I. It would seem that the stratum corneum should be easily disrupted to corneocytes by the extraction of intercellular and membrane lipids, which are considered to play an important role in cell-to-cell attachment (6). However, the disruption of stratum corneum did not occur at all in the lipid solvents. Furthermore, the effects of lipid solvent treatment on subsequent stratum corneum disruption was investigated. After the stratum corneum was immersed in ethanol for a fixed time interval at 25°C, it was transferred to the aqueous solution of SDS/C_{12}DMAO (2:8) mixture (0.1 M/l) and kept at 50°C for 24 hrs. The corneocytes were then counted. The results are shown in Table II. Surprisingly, it was apparent that the counts of corneocytes decreased with increasing pretreatment time in ethanol and that the stratum corneum treated for 5 days was not split into any fragments.

The results of corneocyte counts obtained with the delipidized stratum corneum are shown in Table III. The stratum corneum section treated with lipid solvents did not

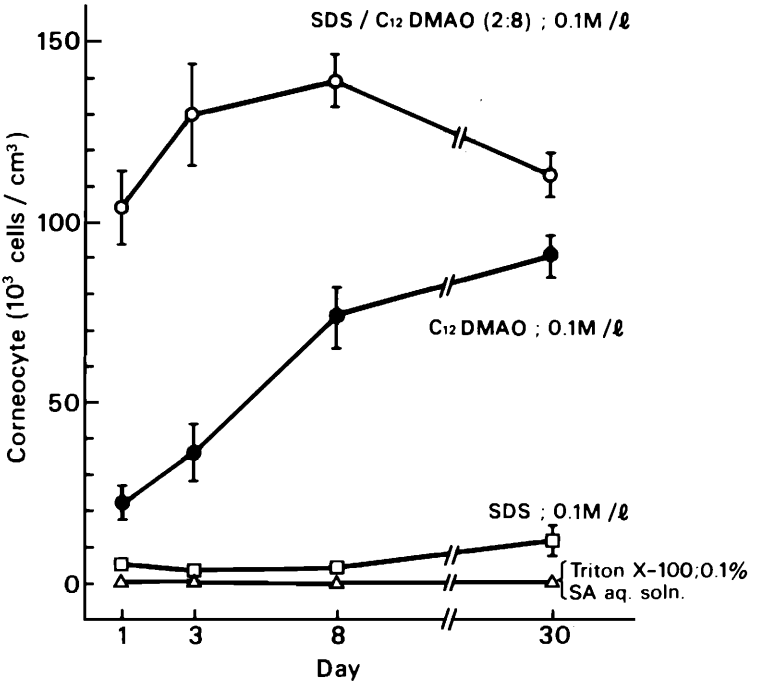

Figure 2. Corneocyte counts (at 25°C) as a function of immersion time in typical surfactant and keratolytic solutions. Results are shown as means and S.E. (n = 8).

change its shape, and only a few corneocytes were observed in SDS/C_{12}DMAO solution. Control samples (treated with distilled water instead of solvent), however, were completely reduced to corneocytes, showing high numbers of cells.

Figure 4 depicts the rheological properties of stratum corneum resulting from immersion in the test solutions. For the samples treated with Triton X-100 or SA, the elasticity values were changed little from the control (distilled water treatment), but the stratum corneum immersed in SDS/C_{12}DMAO (2:8) solution had lower elasticity values than the control. Breaking strength behaved in an almost similar manner to elasticity, and the stratum corneum became markedly fragile due to immersion in aqueous solutions of SDS/C_{12}DMAO (2:8) mixture (statistical significance $p < 0.01$, $n = 4$ compared with control).

DISCUSSION

The present results show that a SDS/C_{12}DMAO mixture markedly reduces the stratum corneum into intact individual corneocytes without mechanical stimulation, with the optimum molar ratio of 0.8 for C_{12}DMAO, for stratum corneum disruption. Previous work by Miyazawa *et al.* (7) found that the surface tension of an aqueous solution of a SDS/C_{12}DMAO mixture has a minimum at a mole fraction of 0.75 for C_{12}DMAO corresponding to the concentration at which stratum corneum disruption is at a maximum. It has also been found that in this mixture a complex forms between the protonated C_{12}DMAO cation and the dissociated SDS anion at pH 9. Furthermore, Epstein *et*

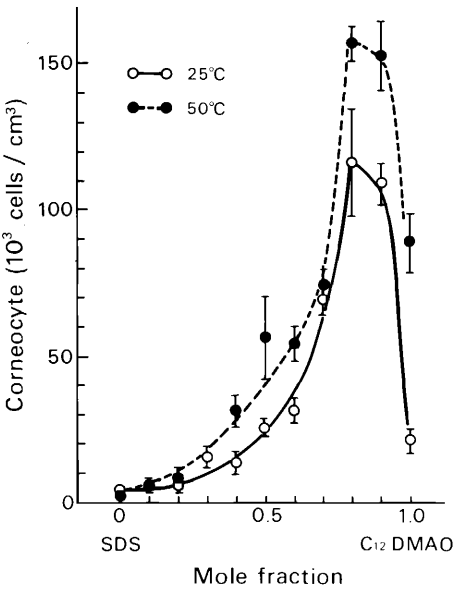

Figure 3. Relationship between corneocyte counts and mole fraction of C_{12}DMAO in SDS/C_{12}DMAO mixture (total concentration; 0.1 M/l). Results are shown as mean and S.E. (n = 8).

al. (8) have reported that the cohesion between corneocytes is increased by ionic interactions. From these results, it can be postulated that this surfactant mixture can uniquely dissolve the intercellular cement materials by its low surface tension and an electrostatic effect. The number of corneocytes released in SDS/C_{12}DMAO solution was almost constant after 1 day of immersion, because the stratum corneum seems to be completely reduced to individual corneocytes in 24 hrs. The surface area of corneocytes and the number of layers in the stratum corneum used in this experiment were roughly estimated to be 1×10^3 μm^2 and 15 layers, respectively. If the sample of stratum corneum (10 mm × 10 mm) was completely reduced to individual cells in 10 ml of a test solution, the corneocyte count would be $(10^4 \times 10^4/10^3) \times 15/10 = 1.5 \times 10^5$ cells/cm^3. This value is comparable to that obtained in our experiments.

Table II
Number of Corneocytes Released From Ethanol-Treated Stratum Corneum in 0.1 M/l Solution of SDS/C_{12}DMAO (2:8) Mixture

Treatment time with ethanol	Corneocytes
1 hr	182 ± 10
3 hr	131 ± 9
8 hr	104 ± 12
24 hr	71 ± 11
3 days	29 ± 6
5 days	9 ± 3

Stratum corneum was immersed in an aqueous solution of SDS/C_{12}DMAO mixture at 50°C for 24 hrs after treatment with ethanol at 25°C. Results are expressed as cells/cm^3 × 10^{-3} and shown as mean ± S.E. (n = 8).

Table III
Effects of Lipid Solvent Treatments on the Number of Corneocytes Released From Stratum Corneum Immersed in 0.1 M/l Solution of SDS/C_{12}DMAO (2:8) Mixture

Treatment	Immersion time in SDS/C_{12}DMAO (at 50°C)			
	1 Day	3 Days	8 Days	30 Days
Ether	1.17 ± 0.42	1.17 ± 0.49	3.17 ± 0.68	1.00 ± 0.49
Ethanol	0.25 ± 0.18	0.58 ± 0.34	0.83 ± 0.34	0
Chloroform/methanol (2:1) mixture	0.92 ± 0.67	3.42 ± 1.92	4.00 ± 1.93	0.83 ± 0.51
Control	104 ± 10	130 ± 14	139 ± 7	113 ± 6

Stratum corneum was treated with lipid solvents at 25°C for 6 days before immersion in aqueous solution of SDS/C_{12}DMAO mixture. Results are expressed as cells/cm^3 × 10^{-3} and shown as mean ± S.E. (n = 8 − 10).

Recently papers have been published which describe the interrelation between desquamation and polar lipids (9,10). According to these authors, lipids, especially cholesteryl sulfate, serve as an intercellular cement in the stratum corneum. This lipid is reported to be removed from stratum corneum by exhaustive extraction with chloroform/methanol (2:1) mixtures (10). In our experiments, however, lipid solvents could not disrupt the stratum corneum into corneocytes. Furthermore, stratum corneum treated with lipid solvents was not fragmented to corneocytes at all, even by a solution of SDS/C_{12}DMAO (2:8) mixture. These results suggest that there may be important factors other than cholesteryl sulfate in determining the intercellular binding behavior of horny cells. Residual annular desmosomes, interdigitation of keratinocytes, and the physical state of the extracellular lipid are probably all of some importance in main-

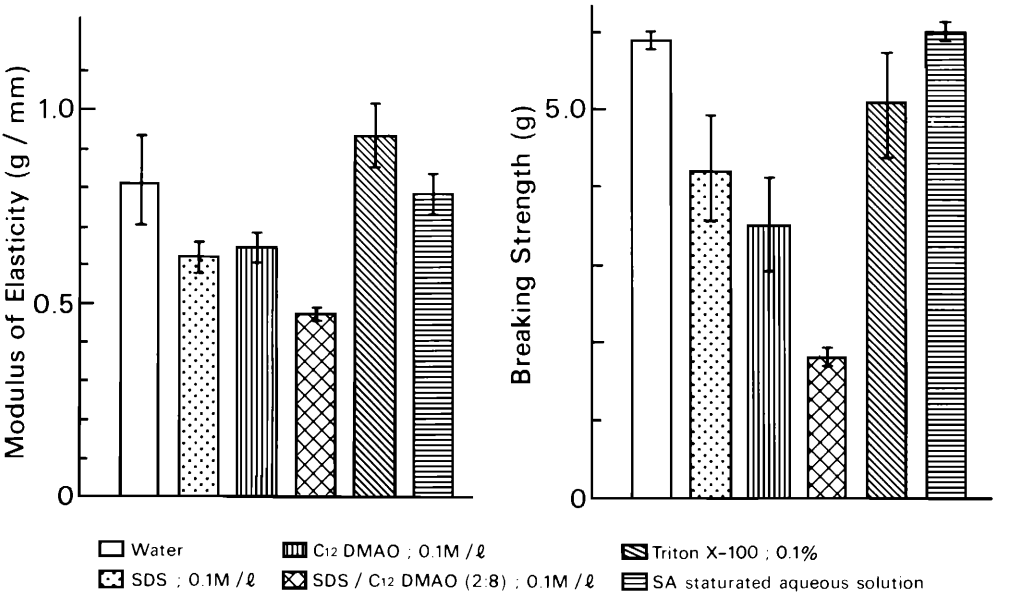

Figure 4. Rheological properties of stratum corneum immersed in test solutions at 25°C for 2 hrs. Results are shown as mean and S.E. (n = 4).

taining the structure of the stratum corneum. It is still not clear why the treatment with lipid solvents prevents the fragmentation process. However, presumably, solvent treatments alter either the physicochemical nature of the intercellular cement material or the intercorneocyte bonds or both of these so that the solvent-treated stratum corneum is not fragmented to corneocytes when immersed in the SDS/C_{12}DMAO mixture.

The biomechanical properties of stratum corneum directly relate to the mode of intercellular attachment. Scanning electron and conventional microscopic analysis of stratum corneum samples fractured under a load indicates that the samples predominately fracture within the intercellular junctions rather than intracellularly (11). Agach *et al.* (12) have also stated that the reduction of breaking strength of stratum corneum is due to an alteration of the links between cells and is related to the damage of cell cohesion devices. As a result of these findings, stratum corneum breaking strength seems to provide a useful parameter for studying cellular cohesive forces. From our experimental results, it was shown that a solution of SDS/C_{12}DMAO (2:8) mixture markedly reduced the breaking strength of stratum corneum. Therefore, it may be postulated that the SDS/C_{12}DMAO mixture decreases the cohesive forces between corneocytes by causing the dissolution of intercellular cement or by altering the links between cells. Though the mode of action of the solution of SDS/C_{12}DMAO mixture on stratum corneum remains obscure, it appears to be different from that of SA (typical keratolytic agent) which shows no effects on the mechanical properties of stratum corneum and does not cause its fragmentation to individual corneocytes. Studies are in progress to more precisely define the important components in stratum corneum cell adhesion by examining the materials extracted.

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