Organization of Stratum Corneum Lipids in Relation to Permeability: Influence of Sodium Lauryl Sulfate and Preheating

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The role of the structural organization of intercorneocyte lipids in the barrier function of human stratum corneum was evaluated by treatment with heat and sodium lauryl sulfate. Measurement of transepidermal water loss in treated samples was used to quantify variations in stratum corneum permeability. Thermodynamic transition of lamellar lipids and their degree of organization were evaluated by differential scanning calorimetry and small-angle X-ray diffraction, respectively. Progressively preheating stratum corneum samples from 75°C to 90°C increased stratum corneum permeability to water vapor, while the fusion temperature of lamellar lipids and the intensity of the X-ray diffraction peaks of the polar lipids decreased. Sodium lauryl sulfate induced similar variations of these three parameters. These results support the hypothesis that, in addition to the chemical nature of intercorneocyte lipids, their structural arrangement and thermodynamic properties play an important role in the barrier function of the stratum corneum to water vapor.

KEY WORDS: stratum corneum; lipid organization; permeability; sodium lauryl sulfate; preheating.

INTRODUCTION

The role of the stratum corneum (SC) in skin permeability has been recognised and the importance of intercellular lipids is now accepted (review in ref. 1). Further, thermotropic properties of this lipid matrix and its structural organisation have been described and their influence on diffusion phenomena discussed (2-3). However, the relationship between lipid organization and permeability of human SC remains unclear.

One technique commonly used to explore thermal properties is differential scanning calorimetry. Four transitions were detected for hydrated human SC (4-7). The first, ($T_{m1} \approx 37°C$) and second transition ($T_{m2} \approx 70°C$) were ascribed to gel-liquid transitions, because of their reversibility and on the basis of lipid extraction experiments (5). The third transition ($T_{m3} \approx 85°C$), partly reversible and sensitive to hydration, was ascribed to a gel-liquid transition affected by protein and the fourth transition ($T_{m4} \approx 110°C$) is due to the denaturation of the intracellular keratin.

As high hydration level and penetration enhancers such as laurocapram decrease the melting temperature T_{m2} and enhance SC permeability, several investigators assume that this phenomenon is associated with barrier function alteration (4, 5, 7). Other treatments influence the lipid melting temperature. Golden et al. observed that reheating the sample for a second scan shifts the Tm2, and introduced the term lipid fluidity effects to describe the enhanced motional freedom of the hydrocarbon chains, seen in the Infra-Red spectrum, associated with the decrease of the temperature transition T_{m2} in porcine SC when certain fatty acids are applied. Similarly surfactants like sodium lauryl sulfate (SLS) decrease lipid melting temperature (5) and increase water diffusion. Do these treatments modify lipid structural organisation?

X-ray diffraction is used to study this organisation (6-11). Several hypothesis about the interpretation of SC diffraction pattern can be considered. White et al. detected a lamellar period of 13.1 nm in small angle diffraction pattern of hairless mouse SC (9). Garson et al. described a human SC pattern, with a synchrotron, at wide and small angles X-ray scattering (10). An intense pattern at 6.5 nm and a more diffuse one about 4.6 nm were detected and attributed to intercellular polar lipids. A third period at 4.1 nm is sometimes observed, and could correspond to neutral lipids. Bouwstra et al. confirmed the lamellar spacing at 13.1 nm detected by White et al. (9). The intense pattern at 6.5 nm, described by Garson et al. is readily observed (10). The diffuse pattern at 4.6 nm is not easily detected because it forms a shoulder on the scattering curve (11). Penetration enhancers like laurocapram decrease the general pattern intensity and introduce lipid disorder (6).

The influence of preheating, laurocapram, or SLS treatments on the transcutaneous penetration was investigated by different techniques. Preheating from 75°/80°C improved alcanol permeability in neonatal rats (12). Enhancement of tritiated water vapor diffusion through porcine SC was described by Golden et al. from preheating at 75°C (4). Similarly, SLS treatment enhances in vitro skin permeability (13) and in vivo TEWL (14). The mechanism of this permeability alteration is still under discussion (15-16).

This survey on the barrier function in relation to the structural organisation of the lipids shows that a link has been demonstrated between these phenomena but under various experimental conditions. Nevertheless, no convincing arguments were proposed about the correlation of lipid thermotropic transitions, structural organisation and water vapor permeability. Our aim was to define the interrelationship of these parameters by quantifying them in the same samples with the same treatments. Thus, this study focuses on the effect of controlled preheating and SLS treatment, by combining DSC, X-ray diffraction and transepidermal water loss measurements (TEWL) on human stratum corneum in vitro.

MATERIALS AND METHODS

Stratum Corneum

SC was isolated from human skin (breast or abdomen)

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obtained during surgery. Epidermis was obtained by immersing whole skin, in plastic bag, in a distilled-water bath at 56°C for two minutes. Treatment with 0.05% trypsin (Prolabo, Paris, France) in a pH 7.9 Tris/HCl buffer for 60 minutes allowed isolation of the SC (17). After rinsing with distilled water, samples were stored in a dessicator. Some control samples were prepared from epidermis separated from the dermis in ammonia vapours.

Treatments

Thermal Treatment

For TEWL measurements, samples were heated from 70°C to 95°C in increments of 5°C, during 30 minutes, in a humidified oven (a water recipient is placed in the oven) in order to limit dehydration. The sample is then cooled. TEWL was measured 24 hours after return to room temperature, in accordance with the measurement conditions of the device used for this purpose.

For the DSC studies, thermal treatment was performed inside the calorimeter. Samples were hydrated at about 10%. After the control thermogram had been recorded, the sample was maintained in the oven during 30 minutes at the desired temperature, and then cooled. The effects of successive treatments (80°, 85°, 90°, and 95°) were recorded during the next thermogram.

Experimental conditions were identical for the X-ray diffraction and TEWL studies. We studied the effects of the preheating condition which most strongly modified lipid stability. The influence of preheating temperature was always evaluated at room temperature, after the sample had been cooled.

SLS Treatment

SLS was purchased from Fluka (Chemika-Biochemika, Switzerland, >98% purity). The concentration of aqueous solutions of SLS used were 0.1%, 0.2%, 0.3%, 0.5%, and 1%, a range that included the critical micellar concentration (CMC) for SLS (0.24%) (18). Samples were immersed for 2 hours, then rinsed and dried.

Measurement of TEWL

Disks of SC (1 cm in diameter) were placed on stainless-steel cells containing water. After allowing them to stabilize for 24 hours in a temperature- and humidity-controlled environment, the quantity of water vapor diffusing through the sample was measured with a Servomed EP1C vaporimeter (Stockholm, Sweden) (19). Passive diffusion is governed by Fick's law, which can be used to determine the permeability constant (Kp) of water vapor in the SC as a function of the measured TEWL. We calculated the ratios for the Kp of treated SC samples to control values (Kp_t/Kpt_o). In the SLS study, control samples previously immersed in distilled water for 2 hours were analyzed in order to determine the effects of the solvent.

Differential Scanning Calorimetry

A Perkin Elmer DSC7 calorimeter was used. SC samples of about 5 mg were placed in stainless steel pans (B182901) and heated at a rate of 5°C/min. We defined the temperature transition as the top of the peak transition because another way (onset) was not always possible. We followed variations of the lipid fusion temperature (Tm2) around 75°C, as a function of tensioactive concentration and thermal treatments. The absolute values of DTm2 ($DTm2 = Tm2_{o(control)} - Tm2_{t(treated)}$) were plotted.

X-Ray Diffraction

The source used is the synchrotron of the LURE (University of Paris Sud). Diffraction experiments were carried out on the D43 station, using a collimated and monochromatic incident beam (with a 0.5 mm cross section and a wavelength $\lambda = 0.149$ nm respectively). Detection was obtained on a sensitive film (Kodak DEF-5). We used a sample geometry in which the incident beam was parallel to the SC sheets. The film was placed perpendicularly to the incident beam, at a distance of 390 mm from the object. This rather long distance had been chosen to correctly observe diffraction patterns from lipid stacks (10).

The diffraction angle 2Θ (angle between the incident and diffracted beam) is related to the equidistance d of the reflecting stack of planes by Bragg's formula $2d\sin\Theta = n\lambda$, where λ is the wavelength and n the order of the diffraction peak. The scattering vector $Q_n = 2\pi n/d$ relates the position of the n_{th} order diffraction peak and the repeat distance d.

Peaks located at 6.5 nm, 4.6 nm and 4.1 nm have been observed and their profiles measured with a densitometer Camag TLC II. Profiles were shifted when necessary for clarity.

RESULTS

Temperature Influence

TEWL measurements revealed a water vapor Kp (permeability constant) of the control sample of $(0.50 \pm 0.03) \times 10^{-3}$ cm/h (mean ± standard deviation). This value agrees with the literature (20).

Figure 1 presents the variations in SC permeability constant (Kpt/Kpt_o ratio), as a function of the preheating temperatures. The permeability of SC samples heated to 70°C and cooled, 24 hours before TEWL measurement, was the same as the control. Above 75°C, and up to around 90°C,

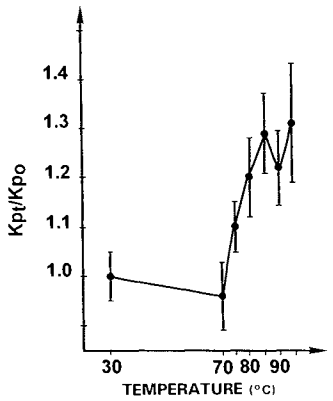

Figure 1: Variation of the Kp_t/Kpt_o ratio (Kp: permeability constant) as a function of the preheating (mean ± SEM).

permeability progressively increased before tending to stabilize. The influence of these thermal treatments, measured after the sample had returned to room temperature, produced an 30% increase of SC water vapor permeability.

Figure 2 represents the variations of DTm2 (absolute mean values). The decrease in the Tm2 of lamellar lipids started at the pretreatment temperature of 80°C, accentuated until 90°C, and then stabilized at 95°C. This decrease was 5 or 6°C, which corresponds to significant lipid modification. X-ray diffraction studies showed that thermal treatment around 90°C, which strongly modify the SC barrier function and the thermal transition of lamellar lipids, disorganized SC lipid structure. The features located at 6.5 nm and 4.6 nm were less clear and less intense than in the control pattern. The third diffraction arc at 4.1 nm disappeared. These variations indicated the existence of a major bilayer lamellar alteration. The densitometry profile presented in Figure 3 summarizes these observations.

SLS Influence

As shown in Figure 4, the increase in permeability began at 0.1% SLS, peaked at 0.5% SLS (a higher value than the critical micellar concentration) and remained stable thereafter. At 0.5% SLS, the permeability of SC was increased twofold.

Using DSC, we found that the transition temperature of lamellar liquids (Tm2) shifted strongly, starting at 0.3% SLS, and appeared to stabilize thereafter (Fig. 5). These variations indicated lipid modification at SLS concentrations above the CMC. The lipid fusion peak shifts but the presence of transition peaks indicated that lipid extraction was minimal.

The effects of these treatments on the supra-molecular organization of lipid bilayers are evident in the densitometry profiles (Fig. 6). Above 0.3% SLS, the peaks around 6.5 nm and 4.5 nm were blurred and much less intense, indicating that SLS has a disrupting effect that is most intense at concentrations around 0.5% SLS. In addition, as the SLS concentration increased from 0.3% to 0.5%, the thin, fine arc of the neutral species observed at 4.1 nm became wider and less clear, and shifted first to 4.00 and then to 3.90 nm. These changes in the structural organization of lipids were reversible, after prolongated rinsing, indicating that SLS disorganizes but did not extract SC lipids.

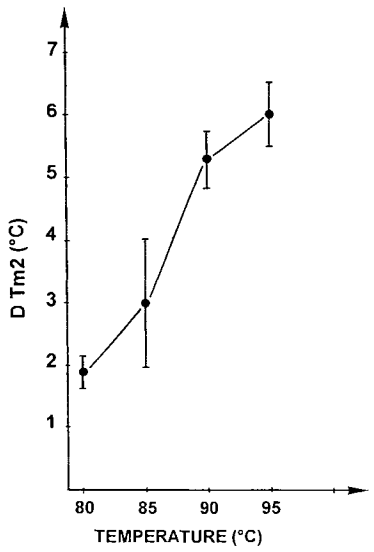

Figure 2: Variations of Tm2 (Tm2: lipid melting temperature) (DTm2 = $Tm2_t$ – $Tm2_o$, absolute values) as a function of the preheating (mean ± SEM).

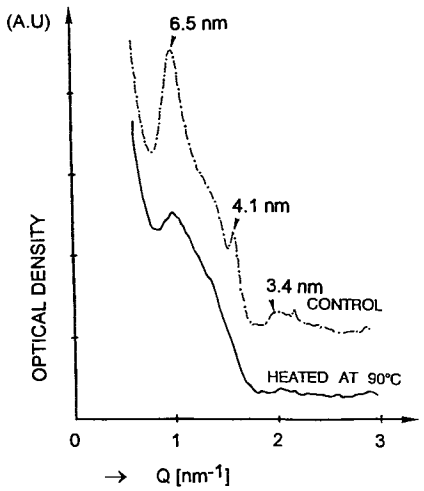

Figure 3: Densitometry profiles from X-ray diffraction patterns: control, SC heated at 90°C, plotted as a function of the scattering vector Q_n. $Q_n = 2\pi n/d$ relates the position of the n_{th} order diffraction peak and the repeat distance d of lamellae arrangement. Assuming the main peak is the first order, d = 6.5 nm.

DISCUSSION

The SLS or heat treatments of SC samples had several effects on the measured properties. The diffusion of water vapor increased, thermal transition of the lipids decreased and the SAXS diffraction pattern was disturbed. What is the relation between the observed alteration in barrier function and the thermodynamic and structural parameters?

The DSC investigations presented here reveal that both treatments decrease lipid melting temperature. The preheating results, figure 3, show that, a preheating at about 85/90°C, lipid transition amplitude decreases. Thermal treatments offer the advantage of modifying a medium without introducing a new component. Our findings show that lipid

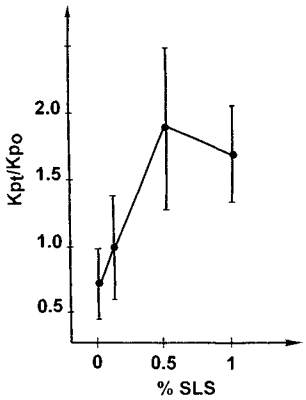

Figure 4: Variation of Kp_t/Kpt_o ratio (Kp: permeability constant) as a function of the SLS concentration (mean ± SEM).

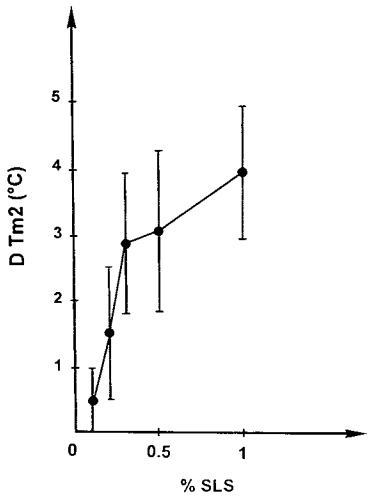

Figure 5: Variation of Tm2 (Tm2: lipid melting temperature) (DTm2 = $Tm2_o$ – $Tm2_t$, absolute values) as a function of the SLS concentration (mean ± SEM).

modification can be induced without lipid composition variation.

The SLS treatments introduced a similar decrease of lipid melting temperature from the CMC. This variation is comparable to the laurocapram effect, but with a decreased amplitude (5,7). This lipid modification has been described by Goodman et Barry, but they did not report the importance of the molecular form of the tensioactive form (monomer or micellar) (5). Further, we note that the peak corresponding to the lipid melting is always detected on the treated sample thermograms. Thus, intercellular lipids are still present and no major lipid extraction occurred during treatment with this amphiphilic molecule.

The consequence of these thermodynamic variations are appreciated when considering the TEWL measurement; both treatments alter barrier function.

The findings concerning preheatings agree with previous observations: preheating from about 75°C enhances rat SC permeability to alcanols or tritiated water diffusion through porcine SC (4,12). DSC results allowed us to propose lipid modification to explain permeability enhancement. Qualitatively we confirm that preheatings act at about 75°/80°C and that human SC is also affected as porcine or rat samples.

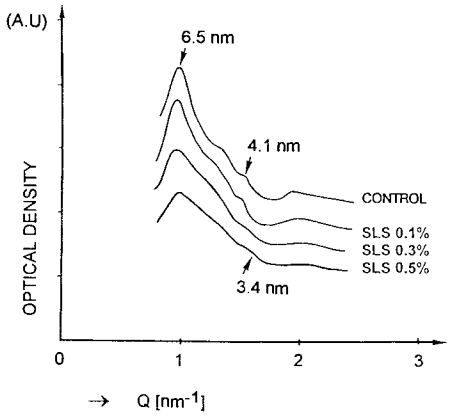

Figure 6: Densitometry profiles from X-ray diffraction patterns of SC as a function of the SLS concentration. The profiles are plotted as a function of the scattering vector Q_n. Assuming the main peak is the first order, d = 6.5 nm.

The results also show that SLS disturbs barrier function in vitro. SC permeability is enhanced twofold by SLS treatments. Similar variations of SC TEWL were previously observed in vitro and in vivo (19). Thus, direct alterations of SC have to be considered. Froebe et al and Lévêque et al, demonstrated that ceramides were not extracted by SLS (16,19). DSC results favor the hypothesis that SLS interacts with intercellular lipids. The observations concerning preheating confirm that modification of their thermotropic properties is sufficient to enhance SC permeability. These considerations lead us to propose that SLS alters barrier function through lipid alteration.

To further this biophysical approach, it is helpful to analyse the structural organisation of intercellular lipids. Preheating at 90°C and SLS treatment from the CMC has a marked effect on the X-ray diffraction pattern. With both types of treatment, the intensity of the peaks at 6.5 nm and about 4.6 nm are strongly attenuated, which clearly indicates intercellular lipid disorganisation. Bouwstra obtained a similar result with azone and interpreted it as a local disorder induced by molecular insertion into the bilayer (7). A similar phenomenon may occur with SLS from the CMC and emphasizes the importance of lipid structural organisation in permeability.

Bouwstra observed that, preheating the SC at 75°C, decreases the intensity pattern, but from 95°C a recrystallization phenomenon appears and a different pattern is observed (11). Our results confirm these observations since preheating at 90°C disorganizes the lipid bilayer.

The pattern at 4.1 nm disappeared after preheating and shifted towards 3.9 nm in presence of SLS. According to Garson it is probably produced by neutral lipid, less implicated in SC permeability (10).

Two of the changes recorded in these experiments, the decrease in transition temperature and the decrease in the intensity of the diffraction pattern, indicated the introduction of a disorder. The results concerning these two different treatments prove that structural disorder must be consider in permeability investigations and that its induction is not a typical property of penetration enhancers. The result obtained for TEWL, the third parameter recorded, confirms that they weaken the barrier function of the SC while preserving the lipid content.

The barrier function of the SC is mainly due to ceramides. The experiments presented here complement this conclusion by demonstrating the importance of lipid organization in their ability to control permeability. Both the structural organization and the chemical nature of these lipids appear important in the SC barrier function.

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